Experimental Use Permits (EUP-99-01 and EUP-99-02
Final Report

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DATA REQUIREMENTS

GDLN 96-12: Efficacy of rodenticides of farm and rangel

AUTHORS

E. B. Spurr, G. D. Lindsey, C. Forbes Perry, and D. Foo

STUDY COMPLETED

June 30, 2003

LABORATORY

U.S. Geological Survey
Biological Resources Division
Pacific Island Ecosystems Research Center
Kilauea Field Station, P. O. Box 52
Hawaii Volcanoes National Park, HI 96718

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STATEMENT OF DATA CONFIDENTIALITY

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

Study Director: David Foote — 19 February 2004

David Foote
U.S.G.S. Biological Resources Division
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Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was not conducted in accordance with the requirements of Title 40, Code of Federal Regulations, Part 160, Good Laboratory Practice Standards, as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs.

Study Director: _____ 19 Feb. 2004

David Foote — Date
U.S.G.S. Biological Resources Division
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Study Director's Management: _____ 2/19/04

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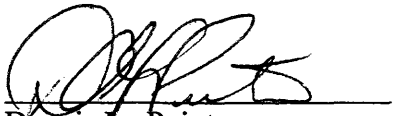
QUALITY ASSURANCE STATEMENT

This study was maintained on the Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station Master Schedule. In order to evaluate the study in terms of compliance with Title 40, Code of Federal Regulations, Part 160, as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs, the study was inspected at different critical periods. The dates of inspections, dates of submission of reports to the Study Director and the Study Director's Management are listed below. The report describes the methods and procedures used in the study, and the reported results accurately reflect the raw data.

Phase Inspected	Inspection Date
Protocol	23 September 1999
In-field Study	18 October 2001
Analytical Chemistry	24 March 2000
Raw Data	16 July 2002
Draft Report	17 March 2003
Final Report	25 August 2003

Quality Assurance Officer:  8/25/03

Dennis La Pointe — Date

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I. ABSTRACT

Rat numbers were monitored in paired 4-ha treatment and non-treatment plots, in both wet and mesic forest sites, before and after hand-broadcast application (simulating aerial application) of Ramik® Green pelletized baits containing 0.005% (50 ppm) diphacinone in the treatment plots. Radio-telemetry revealed that rat numbers in both treatment plots were reduced by 100% within about 1 week. Live-trapping and monitoring non-toxic census bait blocks for signs of rat gnawing indicated a reduction in rat numbers of 98–100% in the 2–4 weeks after the initial bait applications, and generally 70–100% in the 2–4 weeks after subsequent bait applications. However, these two methods did not accurately measure efficacy, because there could have been immigration of rats after bait application. None of the rats captured in the treatment plots 2–4 weeks after bait application had been ear-tagged in the treatment plots before bait application (i.e., they were not recaptures), whereas, on average, 22% of the rats captured in the non-treatment plots were recaptures. This supports the interpretation that the rats that were captured in the treatment plots 2–4 weeks after bait application were immigrants rather than survivors of the treatment. Black Rats (*Rattus rattus*) comprised about 75% of rats captured in the wet forest and 97% of rats captured in the mesic forest. The proportion of Black Rats captured in the treatment plot in the wet forest decreased and Norway Rats (*R. norvegicus*) increased 2–4 weeks after bait application. The age and sex ratios of rats captured in the treatment plots were similar before and 2–4 weeks after bait application. Rat populations in the treatment plot in the wet forest recovered to pre-poison levels within about 8–12 weeks of bait application, presumably by immigration of rats from surrounding areas. Non-target mortality included the House Mouse (*Mus musculus*) and Indian Mongoose (*Hespestes auropunctatus*). No birds were found dead but residues of diphacinone were found in the livers of three species of introduced birds, and also in the mice and mongoose. Based on the success of this and previous studies, we recommend that an aerial-broadcast application of Ramik® Green baits be made in a conservation area in Hawaii to evaluate the effectiveness of this technique for the control of rat populations.

II. INTRODUCTION

A. BACKGROUND

Alien small mammal predators have had devastating impacts on insular environments worldwide (Atkinson 1977, 1985; Buckle and Fenn 1992; Moors et al. 1992; Seto and Conant 1996). In Hawaii[1], evolution of the flora and fauna occurred in a relatively high degree of isolation, and native plants and animals are unusually susceptible to selection pressures from non-native animal species. Today, native Hawaiian wet forests harbor much of the remaining endemic biological diversity. Hawaiian mesic forests cover less area than do wet forests and have been much disturbed by human activities, but those in protected areas support a diversity of native woody plant species. The impact of introduced predators on forest health and ecosystem properties in these habitats is poorly understood. Four species of introduced rodents, the Black Rat (*Rattus rattus*), Polynesian Rat (*R. exulans*), Norway Rat (*R. norvegicus*) and House Mouse (*Mus*

[1] Hawaiian names are spelt without diacritical marks except in literature citations and attached documents.

musculus) are found in a variety of habitats in Hawaii, from sea level to 3050 m elevation (Stone 1985; Tomich 1986; Lindsey et al. 1999). These rodents, together with the introduced Feral Cat (*Felis catus*) and Indian Mongoose (*Hespestes auropunctatus*) inhabit forest habitat in varying degrees of sympatry with native Hawaiian forest birds, plants, and invertebrates (Tomich 1986; Sugihara 1997; Stone and Pratt 2002; USGS/BRD unpubl. data).

Depredation of eggs, nestlings, and adult birds by introduced mammalian predators has been widely postulated as a leading cause of the accelerated decline and extirpation of endemic Hawaiian avian species and as a major factor limiting present populations of endangered forest birds (Atkinson 1977, 1985; Berger 1981; Scott et al. 1986). In addition, rats prey on native Hawaiian tree snails (*Achatinella* spp.) (Hadfield et al. 1993) and insect larvae (Sugihara 1997). Rats may also compete for food with the Hawaiian Crow (*Corvus hawaiiensis*) and Omao (*Myadestes obscurus*) (Scott et al. 1986), and with some endemic insectivorous bird species such as the Akiapolaau (*Hemignathus munroi*) and Hawaii Creeper (*Oreomystis mana*) that specialize on large conspicuous invertebrates (Stone and Scott 1985).

The size, arboreal behavior, and nocturnal habits of Black Rats make them the greatest rodent threat to native forest birds. Both Black and Polynesian Rats are also known predators of ground and burrow nesting birds (Baldwin 1945; Johnson 1945; Kepler 1967; Woodward 1972; Berger 1981; Tomich 1986). Norway Rats are generally restricted to cropland and areas inhabited by humans, and are uncommon in forest habitats (Tomich 1986; USGS/BRD unpubl. data).

Fruits and seeds of many endemic plant species are also susceptible to predation by rats. Rats are considered immediate and significant or potential threats to approximately 90 of the 97 species of native lobelioids tracked by the U.S. Fish and Wildlife Service as endangered, threatened, or proposed species of concern. Identified impacts include bark-girdling, seed-predation, and/or limiting fruit production (U.S. Fish and Wildlife Service 1996, 2002; IUCN 2002). In a study carried out in wet montane forests on the island of Maui, Sugihara (1997) reported a high frequency of fruits and seeds of native plants in rat stomachs; plant species identified included *Rubus hawaiiensis*, *Coprosma* spp., and *Pittosporum* spp. Early reports of rat damage in native wet forests included observations of predation on fruits and seeds of the indigenous liane Ieie (*Freycinetia arborea*) (Perkins 1903) and endemic Loulu palms (*Pritchardia* spp.) (Beccari and Rock 1921).

The impact of rats on endangered plants in wet forests is not well studied. In mesic forests of Hawaii Volcanoes National Park, Black Rats damage flowers, fruit, seeds, and bark of the endangered Hau Kuahiwi tree (*Hibiscadelphus giffardianus*) (Baker and Allen 1978). Bark-stripping and seed predation have also been noted on other mesic forest tree species, including Olopua (*Nestegis sandwichensis*), Pilo (*Coprosma rhynchocarpa*), Koa (*Acacia koa*), Hoawa (*Pittosporum hosmeri*), Sandalwood (*Santalum paniculatum*), and Ae (*Zanthoxylum dipetalum*) (Russell 1980; Scowcroft and Sakai 1984; Cuddihy and Stone 1990).

Ebenhard (1988) concluded that the Feral Cat was the most dangerous predator ever introduced to islands by man, and cited 38 known or probable cases where cats have seriously affected the abundance of prey populations. The role of Feral Cats as predators in Hawaii is poorly known, but several findings suggest that they may be important predators of native birds. Snetsinger et al. (1994) found remains of five banded birds in 30 cat scats collected near Puu Laau on Mauna Kea, even though only a small percentage of birds in the area were banded (USGS/BRD unpubl.

data). Van Riper (1980) watched a Feral Cat attack and eat a brooding female Palila (*Psittirostra bailleui*), and van Riper (1978) found partial remains of Amakihi (*Hemignathus virens*) and Elepaio (*Chasiempis sandwichensis*) in the stomachs of a mongoose and two cats collected at Puu Laau. Richardson and Woodside (1954) thought that Feral Cats were serious predators on nesting Dark-rumped Petrels (*Pterodroma sandwichensis*) on Mauna Kea.

Mongooses are not normally arboreal and are believed to have poor tree-climbing abilities. However, they are adaptable predators and are known to take eggs, young, and adults of eight species of Hawaiian birds Federally listed as endangered (Stone et al. 1995). They may also occasionally kill forest birds which feed on or near the ground or fledglings which spend some time on the ground before becoming proficient in flying.

There are only two methods (trapping and toxicants) available for controlling rats affecting native animal and plant populations in forested areas of Hawaii. Trapping can be an effective short-term nonchemical means of controlling rats in small or limited areas. Three products containing diphacinone (0.005% or 50 ppm), a first-generation anticoagulant, in peanut butter or fish flavors, have a special local needs registration in the State of Hawaii under section 24(c) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for use in bait stations against rats in offshore islands, forests, and other noncrop areas. The fish-flavored products are also registered for use against Indian Mongooses. However, trapping and use of bait stations are labor intensive and impractical for controlling predators over large conservation areas. Studies at Hakalau Forest National Wildlife Refuge have demonstrated that, while the use of diphacinone bait blocks placed in bait stations was effective in reducing Black Rat populations, Polynesian Rats appeared reluctant to accept the bait in its present formulation or distribution method (Nelson et al. 2002). However, all Polynesian Rats ate placebo baits hand-broadcast on the ground in Waiakea Forest Reserve (Dunlevy et al. 2000).

Rats are highly sensitive to anticoagulant toxicants. Multiple feedings on diphacinone baits are required and most rat mortality generally occurs within 4–9 days (Swift 1998). Based on standard laboratory bioassays, Swift (1998) recommended minimum exposure periods and bait amounts of 7 days and 37.5 g of 0.005% diphacinone bait (Ramik® Green) for Black Rats and 6 days and 30.0 g for Polynesian Rats for effective control of wild rats in Hawaiian ecosystems. A sublethal dose does not result in poisoning symptoms, eliminating the development of bait shyness. The LD_{50} (lethal dose of chemical required to kill 50% of the population) is 0.3–7 mg/kg for rats, 3.0–7.5 mg/kg for dogs (*Canis familiaris*), 14.7 mg/kg for cats, 150 mg/kg for pigs (*Sus scrofa*), 50–300 mg/kg for mice (Extoxnet 1993; IPCS 1995), and 0.18 mg/kg for mongooses (Keith et al. 1990).

Keith et al. (1990) fed diphacinone (0.6 mg and 1.5 mg) to pigs for short periods (2–5 days). All pigs survived and, when sacrificed, symptoms of toxicosis were not evident at necropsy. No diphacinone residues were found in muscle tissues but low levels (0.54–0.83 ppm) were found in liver samples. They noted that this is well below the therapeutic (5 mg) dose for humans. Even at the highest level, more than ten tons of liver would need to be consumed to attain the human therapeutic dosage. During baiting for mongoose control, a pig killed at the study site by researchers showed no residual diphacinone after laboratory analysis (Keith et al. 1990).

Birds seem relatively tolerant to diphacinone; the LD_{50} for the Bobwhite Quail (*Colinus virginianus*) was 1,630 mg/kg and the Mallard Duck (*Anas platyrhynchos*) was 3,158 mg/kg

(IPCS 1995). The LC_{50} (lethal concentration required to kill 50% of the population) was 4,485 mg/kg for Bobwhite Quail and >10,000 mg/kg for Mallard Ducks in 8-day dietary studies (IPCS 1995).

In secondary toxicity studies, Mendenhall and Pank (1980) observed no signs of intoxication in two Barn Owls (*Tyto alba*) that consumed diphacinone-killed rats over a 10-day period. In another test, three Great-horned Owls (*Bubo virginianus*) and one Saw-whet Owl (*Aegolius acadicus*) were fed two diphacinone-killed mice daily for 5 days. Three owls died within 14 days, suggesting that a potential hazard could exist under some field conditions (Mendenhall and Pank 1980). Savarie et al. (1979) fed meat containing 2.7 ppm diphacinone to Golden Eagles (*Aquila chrysaetos*) in laboratory tests for 5–7 days. All eagles survived but all showed varying degrees of toxicity before recovery. These investigators suggested that a secondary hazard exists for animals that feed repeatedly on contaminated tissue. Under field conditions, however, contaminated prey may not be accessible to predators or scavengers (see below) and alternate foods are likely to be available, reducing the possibility of secondary poisoning.

Lindsey and Mosher (1994) assessed the secondary hazard potential of diphacinone to raptors, particularly the endangered Hawaiian Hawk or Io (*Buteo solitarius*) and the Hawaiian Owl or Pueo (*Asio flammeus*), within forested areas. Results from their study suggested that hazards to avian predators from baiting with 50 ppm diphacinone bait would be minimal. Kill-trapped dead rats were located rapidly (average 2.9 days) by mammalian scavengers, and raptors did not appear to recognize dead rodents lying on the forest floor as food items. Rats moving above ground during the day, before and after consuming diphacinone bait, remained under cover, minimizing their exposure to avian predators. Because of the short duration that diphacinone-contaminated rats would be available for scavenging and the availability of a wide range of prey, the potential risk for injury or death for Hawaiian Hawks or Hawaiian Owls as a result of poisoning from diphacinone would be minimal.

The non-target secondary poisoning hazard resulting from the use of anticoagulants is also reduced by the delay in death of the target species, which allows time for the cleaning of the gut content, metabolism, and excretion of the toxicant (Godfrey 1985). Cox and Smith (1992) showed that intake of food declined rapidly in anticoagulant-treated, caged *Rattus norvegicus*. Presumably, bait consumption would also decline. Hooker and Innes (1995) reported that Black Rats poisoned with the anticoagulant brodifacoum maintained normal nocturnal movements and showed no nest change between dawn and dark, suggesting no daytime movements. Most rats died in their nests or under cover, suggesting that few rats dying of anticoagulant poisoning would be found in the open (Lindsey and Mosher 1994; Hooker and Innes 1995).

Over the past two decades, feral ungulates and many alien plant species have been successfully eliminated or reduced in Special Ecological Areas (SEAs) within Hawaii Volcanoes National Park (HAVO). The results of resource monitoring, including population studies of forest birds and rare plants and invertebrates, indicate that control of small mammals is the next major challenge for HAVO resource managers. Introduced rats, Indian Mongooses, and Feral Cats have contributed to the loss or decline of many native species in HAVO, including 12 threatened and endangered vertebrates (four of which are currently missing from HAVO). At least half of the 19 naturally occurring threatened and endangered plant species of the park, as well as most of the native plants listed as "species of concern", have fleshy fruits or large seeds vulnerable to rats. Rat predation on seeds, fruit, and invertebrates contributes to reduced food resources for

forest birds, decreased populations of insect pollinators, and limited reproduction and spread of some endemic plants. As a consequence, testing multi-species toxicants was ranked as the highest priority for research in the 1997 Resources Management Plan for HAVO.

The broadcast application of rodenticides has been used successfully to control introduced rodents for species conservation and ecosystem restoration in New Zealand (Moors et al. 1992; Towns et al. 1993, 1994, 1995; Innes et al. 1995, 1999; Empson and Miskelly 1999) and could potentially be used in Hawaii. Rat control is considered a high priority for many species and ecosystem restoration plans in Hawaii. The apparent success of New Zealand predator control efforts prompted the formation of a multi-agency Rodenticide Working Group to seek regulatory approval for the use of similar techniques in Hawaii. Diphacinone was the rodenticide selected to pursue for registration because of its effectiveness against rats, low risk to non-target species, and short persistence in the environment.

We proposed to evaluate the efficacy and safety of hand-broadcast application (simulating aerial-broadcast application) of pelletized baits containing 0.005% (50 ppm) diphacinone for rodent control in wet and mesic forest habitats within SEAs in HAVO, to generate supporting data needed for State and Federal regulatory approval of the technique (Appendices 1, 2, and 3). The research was conducted under two State of Hawaii Experimental Use Permits (EUP-99-01 and EUP-99-02) (Appendix 4), with the approval of the U.S. National Park Service (NPS), U.S. Fish and Wildlife Service (FWS), State of Hawaii Division of Forestry and Wildlife (DOFAW), and The Nature Conservancy (TNC) of Hawaii (Appendices 5–8), in coordination with the Hawaii Department of Agriculture. A Biological Opinion prepared by FWS in accordance with section 7 of the U.S. Endangered Species Act concluded that the study was not likely to jeopardize the continued existence of the Hawaiian Hawk (Appendix 9). Additionally, FWS concluded that the rodent control program may be beneficial to other endangered species within the project area. An environmental assessment (Appendix 10) was accepted by the NPS (Appendix 11).

B. OBJECTIVES

The objectives of the original proposal (Appendix 1) with amendments (Appendix 2, amendment 1) and deviations (Appendix 3) were to:

1. Determine the efficacy of hand-broadcast application of 0.005% diphacinone bait for the control of rats in wet forest habitat.

2. Determine the efficacy of hand-broadcast application of 0.005% diphacinone bait for the control of rats in mesic forest habitat.

3. Determine the disappearance rate of hand-broadcast 0.005% diphacinone bait from the forest floor.

4. Monitor the secondary hazard potential of hand-broadcast 0.005% diphacinone bait.

C. STUDY DESIGN

The study design consisted of separate wet forest and mesic forest study areas, each containing a paired treatment and non-treatment plot, in which rat populations were monitored before and after bait application

D. STUDY AREAS

Both the wet forest and mesic forest study areas were within Hawaii Volcanoes National Park (HAVO). The paired treatment and non-treatment plots within each study area were 4 ha (10 acres) in size (200 × 200 m). A 12.5-m × 12.5-m grid was established in each plot, consisting of 17 transects, 12.5 m apart, with each transect flagged with markers at 12.5-m intervals (see Appendix 2, amendment 2).

The wet forest study area was in the southwest corner of the Koa Management Unit within `Ola`a Forest, at approximately 1200 m elevation. This 800-ha unit was fenced in 1990 and has been free of feral pigs (*Sus scrofa*) since 1994. The forest in this section of `Ola`a is composed of an open canopy of scattered large `ohi`a trees (*Metrosideros polymorpha*) with an open understory of mixed native trees and a dense lower layer of tree ferns (*Cibotium* spp.). The tree fern layer is 2–5 m tall and has a dense cover of 80–90%. Ground cover consists primarily of native ferns, shrubs, and sedges, but a few alien plants are also common here, particularly yellow Himalayan raspberry (*Rubus ellipticus*) and banana poka (*Passiflora mollissima*). The treatment plot (HAVO Transect 16) was separated from the non-treatment plot (HAVO Transect 18) by 450 m.

The mesic forest study areas (Kipukas Ki and Puaulu) are ancient kipukas on deep ash soil surrounded by lava of the late prehistoric Keamoku flows. Both kipukas are on the lower slope of Mauna Loa at 1200–1360 m elevation, and are approximately 8 km west of the wet forest study site of `Ola`a Forest. Vegetation of the central part of the kipukas is composed of a tall koa/`ohi`a/soapberry (*Acacia koa/ Metrosideros polymorpha/ Sapindus saponaria*) forest. Ground cover is dominated by native ferns and herbs where the forest canopy is dense, but blackberry (*Rubus argutus*) and alien grasses, such as meadow ricegrass (*Ehrharta stipoides*) and *Paspalum* spp., are common in some areas. Kipuka Ki also contains some Jerusalem cherry *(Solanum pseudocapsicum*). Large patches of open grassland with scattered trees also occur in the kipukas (Mueller-Dombois and Lamoureux 1967). Kipuka Ki was fenced against cattle in the late 1940s, and has been free of feral pigs since the mid-1980s. Kipuka Puaulu was fenced against cattle in the 1930s and has been free of feral pigs since the mid-1960s. The treatment plot (in Kipuka Ki) was 1.5 km from the non-treatment plot (in Kipuka Puaulu).

III. MATERIALS AND METHODS

A. BAITS

The test bait was a fish-flavored, green-colored, pellet formulation of Ramik® Green (HACCO Inc., Madison, WI), nominally weighing 6 g and containing 0.005% (50 ppm) diphacinone (Appendices 12 and 13). Two lots of baits were received; Lot No. 125218 on 8 July 1999 (used

in trials from October 1999 to August 2000) and Lot No. 144548 on 8 and 13 November 2000 (used in trials from November 2000 to December 2001) (Appendix 14). Lot No. 125218 was manufactured on 4 May 1999, and so was 2 months old when received. Lot No. 144548 was manufactured on 16 September 2000, and so was also 2 months old when received. Both lots were stored before use, at ambient temperature in a dehumidified, locked room in Building 216 at HAVO.

The weight, length, and width of 205 baits from the first lot were measured on 29 September 1999 and another 205 baits from the first lot on 20 October 1999, and the weight, length, and width of 24 baits from both lots were measured on 23 May 2001 (Appendix 2, amendment 8).

The diphacinone content of both lots of bait was measured at the time of manufacture, using high-performance liquid chromatography (HPLC) (Appendix 15, Mary Ann Douglas, HACCO Inc., pers. comm.). The diphacinone content of the first lot of bait was measured again in April 2000 (12 months after manufacture), and the second lot of bait in January 2002 (16 months after manufacture) and April 2002 (19 months after manufacture) by Genesis Laboratories Inc. (Wellington, CO), also using HPLC (see Appendix 2, amendment 13, and Appendix 16). The diphacinone content of the second lot of bait was also measured again in May 2002 (when the bait was 20 months old) by HACCO (Appendix 15, Mary Ann Douglas, HACCO Inc., pers. comm.).

B. BAIT APPLICATION

The rate of bait application was 20 lbs/acre (22.4 kg/ha) in each treatment plot. One-half of the bait (10 lbs/acre or 11.2 kg/ha) was hand-broadcast on day 1, and the other half 4–6 days later. This ensured that baits were available to rats over the recommended time period of 10–15 days (Dunlevy et al. 2000).

The baits were hand-broadcast according to the Standard Operating Procedures (SOPs) BRD-15 and BRD-17 (Appendix 17). All personnel involved wore long-sleeved shirts, long pants, boots, latex gloves, and cotton gloves over the latex gloves. Personnel walked along each transect, and every 2.5 m threw single baits about 1, 3, and 5 m to each side. Prior to the initial bait application, all personnel practiced hand-broadcasting placebo (untreated) baits of similar size, at the same application rate, along transects in an open field.

Toxic bait was first hand-broadcast in the wet forest treatment plot (HAVO Transect 16 in `Ola`a Forest) on 7 and 12 October 1999, and in the mesic forest treatment plot (Kipuka Ki) on 27 January and 1 February 2000. Eleven further hand-broadcast "series" (comprising two applications of bait, 4–6 days apart) were made at 2–4-month intervals between then and December 2001 in the wet forest treatment plot, and two further hand-broadcast "series" were made at 3–5-month intervals in the mesic forest treatment plot (see Appendix 2, amendment 8, and Appendix 18). The baits were applied only when the ground was reasonably dry and predictions were for favorable weather conditions over the next 5 days.

C. BAIT DISAPPEARANCE RATE

The disappearance or degradation of baits was monitored after each series of bait applications, according to SOP BRD-12 (Appendix 17). Twenty locations (at least 25 m apart) were randomly selected within the first quarter of each treatment area, and marked with a wire flag (see Appendix 2, amendment 9). One bait was then placed beside each marker. The presence or absence of each bait was recorded at 1–3 day intervals for up to 14 days or until it disappeared or disintegrated (see Appendix 3, deviation 7).

D. RAT ACTIVITY INDICES

Rat activity was monitored before and after bait application using three techniques: radio-telemetry (first hand-broadcast series only), live-trapping, and non-toxic census bait blocks.

Radio-telemetry. Radio-telemetry was carried out according to SOP BRD-10 (Appendix 17). Radio transmitters (Holohil PD-2C, weighing 4.2 g) were fitted to 6 Black Rats (all that could be caught) in the treatment plot and 13 Black Rats in the non-treatment plot in the wet forest, 1 week before the first bait application in October 1999. One rat in the treatment plot and two rats in the non-treatment plot slipped their radio-transmitter collars before bait application, reducing the sample size to 5 rats in the treatment plot and 11 rats in the non-treatment plot in the wet forest. Radio-transmitters were also fitted to 17 Black Rats in the treatment plot and 15 Black Rats in the non-treatment plot in the mesic forest, 1 week before the first bait application in January 2000. The transmitter on one rat in the non-treatment plot stopped functioning and the transmitter on a second rat in the non-treatment plot was recovered before bait application, so the final sample size was 17 rats in the treatment plot and 13 rats in the non-treatment plot in the mesic forest (see Appendix 2, amendment 6).

Radio signals from the radio-collared rats were monitored according to SOP BRD-13 (Appendix 17), using portable receivers (Telonics TR-4) and hand-held two-element directional antennas (Telonics RA-14). Each rat was monitored nightly for 3 consecutive nights immediately before bait application, and nightly for up to 2 weeks after bait application, to determine whether it was alive. A fluctuating, variable-strength radio-signal indicated that the rat was active and alive, whereas a constant, steady radio-signal indicated that the rat was not moving. Each rat not moving during a nightly monitoring session was tracked to its location the next day to determine its fate. The locations of rat nests discovered during the study were recorded, nest material identified, and nest width, depth, and inside diameter measured where possible (see Appendix 3, deviation 3). Nests that could be collected were placed in a plastic ZipLok bag. Woody trees and tree ferns containing rat nests were identified, measured (trunk diameter at 4.5 feet), and the height of the nest in the tree determined where possible.

Dead radio-collared rats recovered during the study were necropsied and examined for green bait in the stomach and intestines, and for hemorrhaging characteristic of anticoagulant poisoning. The carcasses were placed in marked containers, frozen, and sent to Landcare Research (Lincoln, New Zealand) for HPLC analysis of diphacinone residues in their livers (see Appendix 2, amendment 13, and Appendix 19).

For each forest type (mesic and wet), the percentage reduction in the proportion of radio-collared rats surviving in the treatment plot, relative to the non-treatment plot, was calculated from the formula:

% kill = 100 × ((expected number – observed number) / (expected number))

where expected number = number in treatment plot pre-treatment × (number in non-treatment plot post-treatment / number in non-treatment plot pre-treatment), and observed number = number in treatment plot post-treatment.

The effect of bait application on the survival of radio-collared rats in the mesic forest was assessed by a 2 × 2 chi-square analysis of the number of radio-collared rats alive vs. dead, pre- and post-treatment, in the treatment and non-treatment plots. It was not possible to analyze the effect of bait application on the survival of radio-collared rats in the wet forest because too few rats were radio-collared in the treatment plot (see Appendix 3, deviation 4).

Live-trapping. Live-trapping was carried out according to SOP BRD-04 (Appendix 17). A total of 81 Haguruma® traps were placed at 25-m intervals on transect lines spaced 25 m apart within each study plot 2 weeks before the first trapping, and left closed, to allow the rats time to become accustomed to the traps. The traps then remained at the trap locations throughout the study period, with worn out traps replaced when necessary. Two weeks before toxic baits were hand-broadcast within the treatment plots, trap locations within each study plot were pre-baited with shredded coconut 3 days before the traps were opened. Traps were then opened, baited with chunks of coconut, and operated for 4 consecutive nights (maximum 324 trap-nights). The traps were checked daily, and all rats that did not escape (see Appendix 3, deviation 1) were identified to species, sex, and age class (juvenile or adult), and weighed, ear-tagged, and released, according to SOP BRD-09 (Appendix 17). The rats were not anaesthetized while being handled. Traps were opened again in each study plot 2–4 weeks after bait application to determine the efficacy of the baiting (see Appendix 2, amendment 3, and Appendix 18). As before, the traps were pre-baited with shredded coconut 3 days before they were opened. For each forest type (mesic and wet), rat capture rates per 100 corrected trap-nights, pre- and post-treatment in the treatment and non-treatment plots, were calculated following the method of Nelson and Clark (1973).

The percentage reduction in rat capture rates in the treatment plot, relative to the non-treatment plot, in each forest type was calculated from the formula:

% kill = 100 × ((expected capture rate – observed capture rate) / (expected capture rate))

where expected capture rate = capture rate in treatment plot pre-treatment × (capture rate in non-treatment plot post-treatment / capture rate in non-treatment plot pre-treatment), and observed capture rate = capture rate in treatment plot post-treatment.

Rat capture rates pre- and post-treatment in the control (non-treatment) and treatment plots were compared using a generalized linear model (S-Plus for Windows, 2001, Insightful Corporation, Seattle, Washington, USA), adjusting for trap-nights by using log (trap-nights) as an offset term in the model. The ratio of the variance to the mean (a measure of dispersion) was estimated separately for the wet forest and mesic forest, from the "plot by time" interaction in a model of the pre-treatment capture rates in the treatment and non-treatment plots (for the 12 bait

application series in the wet forest and three bait application series in the mesic forest), and these values were used to scale the residual deviances of the generalized linear model before assessing their significance against a chi-square distribution (McCullagh and Nelder 1989) (see Appendix 2, amendment 4).

Non-toxic census bait blocks. Non-toxic census bait blocks were monitored for signs of rat gnawing according to SOP BRD-11 (Appendix 17). Seventy-two non-toxic Census™ bait blocks (gnaw blocks or chew blocks) (Zeneca Inc., DE) were placed at 25-m intervals on the same transect lines as live traps within each study plot, but half-way between the live trap locations (i.e., at 12.5 m, 37.5 m, 62.5 m, etc.). The census blocks were placed on the transect lines 1–3 weeks before and after each series of hand-broadcast application of baits (see Appendix 2, amendment 5, and Appendix 18). They were attached to the ground using a 1-m wire flag inserted through the center of each bait block and into the ground. Bait blocks were examined daily for 2 consecutive days for signs of feeding by rats or other animals (viz., mice, mongooses, birds, and invertebrates). Unfortunately, up to 100% of the bait blocks disappeared in the wet forest non-treatment plot, and up to 69% disappeared in the mesic forest non-treatment plot. It was not possible to determine which animal species was removing the bait blocks, but since rats were the most common species gnawing the remaining blocks, it was assumed that rats were responsible for removing the missing blocks. The only other possibilities were Kalij Pheasants (*Lophura leucomelana*) and Indian Mongooses, but both were relatively rare in the wet forest, and although Kalij Pheasants were more common in the mesic forest, they were not as common as rats. If we had not assumed that rats had removed the bait blocks, we would have had no data when all the blocks disappeared.

The percentage reduction in the number of census blocks gnawed or supposedly removed by rats in the treatment plot, relative to the non-treatment plot, was calculated in the same way as for live-trapping, separately for each forest type (mesic and wet).

The effectiveness of each hand-broadcast bait application series was determined by comparing the proportion of baits gnawed or supposedly removed by rats before and after treatment in the treatment and non-treatment plots using a logistic generalized linear model (in S-Plus for Windows, 2001, Insightful Corporation, Seattle, Washington, USA), with the deviance scaled using the pre-treatment "plot by time" interaction in the same way as for the live-trapping data (but see Appendix 3, deviation 2).

E. MOUSE ACTIVITY INDICES

House Mouse activity was monitored using three techniques: kill-trapping, live-trapping, and non-toxic census bait blocks.

Kill-trapping. In each 4-ha study plot, 42 snap traps (Victor mouse traps) were located at 10-m intervals (two traps per location) along one transect line to estimate mouse densities pre- and post-treatment (see Appendix 2, amendment 7). Trapping was carried out 2–3 weeks before and again 2–3 weeks after the first five bait applications in the wet forest, and the first two bait applications in the mesic forest (Appendix 18). Trapping was also carried out three times at approximately 2-monthly intervals after the first bait application in the mesic forest. Within each trapping session, the traps were baited with coconut chunks, set, and examined daily for 2 days

(but see Appendix 3, deviation 5). The traps were removed after each trapping session, and relocated to a new transect for the next trapping session. Snap trapping was stopped after July 2000 because too few mice and too many non-target species (non-native birds) were being caught (the latter a likely consequence of not placing the traps in protective tunnels).

For each forest type (mesic and wet), mouse capture rates per 100 corrected trap-nights, pre- and post-treatment in the treatment and non-treatment plots, were calculated following the method of Nelson and Clark (1973). The percentage reduction in mouse capture rates, and statistical comparison of mouse capture rates before and after bait application, could not be done because too few mice were caught (see Appendix 3, deviation 6).

Live-trapping. Mouse activity was monitored pre- and post-treatment as a consequence of monitoring rat activity, because mice were also caught in the live traps used for monitoring rats (see above).

Non-toxic census bait blocks. Mouse activity was monitored pre- and post-treatment as a consequence of monitoring rat activity, because mice also left distinctive gnaw-marks on the non-toxic census bait blocks used for monitoring rats (see above).

F. NON-TARGET SPECIES MONITORING

The presence of avian predators (such as hawks and owls) in the study areas was recorded whenever they were observed. Unfortunately, a separate study of radio-marked Hawaiian Hawks was completed before our study began, and there were no radio-marked birds left in our study areas to monitor (see Appendix 1, VII, A, a, 9).

The locations of radio-collared rats dying during the study were recorded to determine if the carcasses were accessible to avian predators.

Four transects (400 m long × 5 m wide) spaced at least 25 m apart in each treatment and non-treatment plot (representing 10% of the area of each plot), were walked to search for non-target species mortality up to 2 weeks before and 2–4 weeks after each series of bait applications (see Appendix 2, amendment 10, Appendix 3, deviation 9, and Appendix 18). Each transect extended 100 m beyond each end of the study plots. The searching took about 4 person hours. In addition, at least 60 person hours were spent in each plot carrying out other activities (such as live-trapping, radio-telemetry, and census bait block monitoring) before and after each bait application. All carcasses found were recorded as to species, weighed, sexed, placed in individually marked containers, frozen, and sent to Genesis Laboratories Inc. (Wellington, CO) or Landcare Research (Lincoln, New Zealand) for HPLC determination of diphacinone residues in their livers (see Appendix 2, amendment 12, and Appendices 16 and 19).

Birds of four introduced species, viz., Kalij Pheasant (*Lophura leucomelana*), Red-billed Leothrix (*Leothrix lutea*), Northern Cardinal (*Cardinalis cardinalis*), and Japanese White-eye (*Zosterops japonicus*), were collected by shooting or mist-netting in the treatment plot 3–6 weeks after the first bait application series in each forest type (see Appendix 2, amendment 11, Appendix 18, and Appendix 20). Birds (viz., Red-billed Leothrix and a Northern Cardinal) caught in kill-traps set for mice were also collected to increase sample sizes. The carcasses were

frozen and sent to Genesis Laboratories Inc. (Wellington, CO) or Landcare Research (Lincoln, New Zealand) for HPLC determination of diphacinone residues in their livers (as above).

Kalij Pheasant activity was monitored pre- and post-treatment as a consequence of monitoring rat activity, because Kalij Pheasants pecked at the non-toxic census bait blocks used for monitoring rats (see Appendix 2, amendment 5).

G. ENVIRONMENTAL CONDITIONS

Rain gauges and minimum/maximum thermometers were placed in the treatment plot in each study area to monitor daily weather conditions. Rainfall and temperature were recorded whenever the study plots were visited, for up to 2 weeks after each bait application series (see Appendix 3, deviation 8).

IV. RESULTS

A. BAITS

The mean weight (and standard error) of two large samples of baits (each $n = 205$) from Lot No. 125218 was 5.8 g (± 0.05 g) (Table 1). The mean weight (and standard error) of a smaller sample ($n = 24$) of baits from both Lot No. 125218 and Lot No. 144548 was 6.2 g (± 0.1 g) and 6.5 g (± 0.1 g), respectively. The mean length and diameter of both lots of baits were also similar.

The diphacinone content of bait from both Lot No. 125218 (used in the first six applications in the wet forest, and in the first two applications in the mesic forest) and Lot No. 144548 (used in the second six applications in the wet forest, and in the third application in the mesic forest) was 51 ppm at manufacture (Table 2, Appendix 16). This is within the Code of Federal Regulations (Title 40, Part 158.175) certified limits of 45–55 ppm. Lot No. 125218 was still within the certified limits 12 months after manufacture. However, Lot No. 144548 was below the certified limits 16 months after manufacture.

B. BAIT APPLICATION

All the allocated bait (200 lb, or 90 kg) was applied (at 20 lbs/acre, or 22.4 kg/ha) to the treatment plots, half on day 1 and half 4–6 days later, in each bait application series (see also Appendix 4). The rate of application of the active ingredient (diphacinone) was 0.008 oz per acre on day 1 and 0.008 oz per acre 4–6 days later, or 0.016 oz per acre in each bait application series.

C. BAIT DISAPPEARANCE AND DEGRADATION

The disappearance rate of baits varied after each application (Fig 1, Fig. 2). For example, in the wet forest, all the monitored baits disappeared from the treatment plot within 7 days in January 2001 (despite little rain), but 15% of the baits remained for at least 24 days in April 2000 (also little rain) (Fig. 1). In the mesic forest, all monitored baits disappeared from the treatment plot within 7 days in October 2000 (after heavy rain), but 50% of the baits remained for at least 14 days in January 2000 (during which time rainfall was less than 2 mm) (Fig. 2).

D. RAT ACTIVITY INDICES

WET FOREST

Radio-telemetry. All five radio-collared rats in the treatment plot were found dead 5–7 days after the initial bait application in October 1999 (Table 3). None of the 11 radio-collared rats in the non-treatment plot were found dead, but two radio-transmitters were found on the ground surface. One was from a rat that had apparently been eaten by a mammalian predator because bits of rat fur and bones were located with the transmitter. It was not possible to determine whether the rat had been eaten before or after it died. The second transmitter was recovered without a rat, and thus the fate of the rat could not be determined. Post-treatment survival of the radio-collared rats in the treatment plot (0 of 5 rats) and non-treatment plot (9 of 11 rats) could not be compared statistically because the sample size was too low (see Appendix 3, deviation 4).

Four radio-collared rats in the treatment plot were found dead 5 days after the initial bait application and one 7 days after (average 5.4 days after). Necropsies of the five rats revealed hemorrhaging typical of diphacinone poisoning (Appendix 21). All had internal hemorrhaging (under skin, around heart, and/or in lungs, liver, bladder, genitals, thoracic cavity, and abdominal cavity) and four also had external hemorrhaging (from mouth, ear, and/or genital region). Four of the rats also had green bait in their stomachs and/or green fecal pellets in their intestines. An average of 3.8 ppm diphacinine was detected in the livers.

Of four nests that were inspected in the treatment plot, all were in Hapuu tree ferns (*Cibotium glaucum*) (Appendix 22). One nest contained three baits, one had one bait, and two had no baits.

Live-trapping. One week before the initial bait application in October 1999, 14 Black Rats were captured in the treatment plot (4.48 rats per 100 trap-nights) and 17 Black Rats in the non-treatment plot (5.58 rats per 100 trap-nights) (Table 4). Two weeks after bait application, 0 rats were caught in the treatment plot and 25 rats (22 Black Rats and 3 Norway Rats) in the non-treatment plot (8.47 rats per 100 trap-nights). The capture rate of rats in the treatment plot 2 weeks after bait application was significantly lower than expected ($\chi^2 = 6.64$, df = 1, $P = 0.01$). Eight weeks after bait application, however, rat numbers caught in the treatment plot exceeded pre-treatment levels (Table 4, Fig. 3).

Repeat bait applications at 2- to 5-monthly intervals over 2 years resulted in rat capture rates in the treatment plot being reduced to zero or near zero 2–4 weeks after each bait application, except in June and August 2000 (Table 4, Fig. 3). Reductions less than 100% relative to the non-treatment plot were not statistically significant at the 95% level of probability (Table 4). In all cases, rat capture rates in the treatment plot increased to near or above pre-treatment levels in 8–

12 weeks after each bait application (Table 4, Fig. 3). Capture rates in the non-treatment plot also fluctuated over time, but not in response to bait application (Fig. 3).

The species composition of the rats caught in the treatment plot 2–4 weeks after bait application was significantly different from that of rats caught before bait application ($\chi^2 = 6.698$, df = 1, $P = 0.010$; Table 5). The main difference was a lower proportion of Black Rats and a higher proportion of Norway Rats after bait application. There was no significant difference in the species composition of the rats caught in the non-treatment plot before and 2–4 weeks after bait application ($\chi^2 = 1.845$, df = 1, $P = 0.174$).

The age and sex ratios of rats caught was similar before and 2–4 weeks after bait application, in both the treatment and non-treatment plots (Table 6). Most rats caught were adult males. The apparent increase from 10.3% juvenile males (25 out of 243 rats) caught before bait application to 19.2% juvenile males (5 out of 26 rats) caught 2–4 weeks after bait application in the treatment plot was not statistically significant ($\chi^2 = 1.896$, df = 1, $P = 0.169$).

None of the rats captured in the treatment plot 2–4 weeks after bait application had been ear-tagged in the treatment plot before bait application (i.e., they were not recaptures), whereas, on average, 22% of the rats captured in the non-treatment plot were recaptures ($\chi^2 = 13.406$, df = 1, $P < 0.001$) (Table 7). However, one rat (an adult female Black Rat) captured in the treatment plot 9 weeks after the August 2000 bait application had been ear-tagged in the treatment plot 7 weeks before bait application, and so had either survived the bait application or been away from the treatment plot while bait was present (Appendix 23). It was not captured in the treatment plot 3 weeks after bait application, and so is not included in Table 7. Four rats captured in the treatment plot 3, 7, 8, and 10 weeks, respectively, after bait application, had been ear-tagged previously in the non-treatment plot, at least 500 m away (Appendix 23). These rats also are not included as recaptures in Table 7 because they were not originally ear-tagged in the treatment plot, but had moved to the treatment plot from the non-treatment plot. Thus, they may not have been present in the treatment plot at the time of bait application.

Non-toxic census bait blocks. Before the initial bait application in October 1999, rats interfered with 29.6% census bait blocks in the treatment plot (26.8% of blocks gnawed by rats plus 2.8% of blocks missing, presumably taken by rats) and 36.1% in the non-treatment plot (31.9% gnawed by rats plus 4.2% missing) (Table 8, Fig. 4). Three weeks after the initial bait application, rat interference had declined to 1.4% in the treatment plot (no blocks missing), but had increased to 87.5% in the non-treatment plot (62.5% gnawed by rats, 25.0% missing). The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was, therefore, 98.0% ($\chi^2 = 378.45$, df = 1, $P < 0.001$). Twelve weeks after the initial bait application, however, rat interference to census blocks had increased to 12.5% in the treatment plot (no blocks missing) and 95.8% in the non-treatment plot (35.2% gnawed by rats, 60.6% missing) (Table 8, Fig. 4).

Repeated bait applications at 2- to 5-monthly intervals over 2 years reduced rat interference to census blocks in the treatment plot to zero or near zero each time, except in May 2001 when it was reduced only to 12.5% (Table 8, Fig. 4). At the same time, rat interference (and missing census blocks) in the non-treatment plot fluctuated from about 93 to 100%. Most bait applications caused a statistically significant reduction in rat interference to census blocks in the treatment plot (Table 8).

MESIC FOREST

Radio-telemetry. All 17 radio-collared rats in the treatment plot were found dead 4–6 days after the initial bait application in January 2000 (Table 9). Of the 13 radio-collared rats in the non-treatment plot, one stopped moving during nightly monitoring and was presumed dead 4 days after bait application in the treatment plot. This rat was not recovered, and was recorded as a natural mortality. Post-treatment survival of the radio-collared rats (0 of 17 rats) in the treatment plot was significantly lower than in the non-treatment plot (12 of 13 rats) ($\chi^2 = 26.154$, df = 1, $P < 0.001$).

Of the 17 radio-collared rats in the treatment plot, seven stopped moving during nightly monitoring 4 days after bait application, four after 5 days, and six after 6 days (average 4.9 days). Six of these rats were recovered dead or dying. One had been partially eaten by a mammalian predator, and so could not be analyzed. Necropsies of the other five radio-collared rats plus one unmarked rat recovered from the treatment plot revealed hemorrhaging typical of diphacinone poisoning (Appendix 21). All had internal hemorrhaging (under skin, around heart, and in lungs, liver, kidneys, genitals, thoracic cavity, and abdominal cavity) and three of the six rats also had external hemorrhaging (from nose and/or genital region). Three also had green bait in their stomachs and/or green fecal pellets in their intestines. An average of 3.4 ppm diphacinine was detected in the livers.

The locations of 14 rat nests in the treatment plot and 13 in the non-treatment plot were identified but none could be inspected because they were high up in Soapberry, Koa, or Ohia trees (Appendix 22).

Live-trapping. One week before the initial series of bait applications in January 2000, 29 rats (28 Black Rats and 1 Polynesian Rat) were captured in the treatment plot (10.86 rats per 100 corrected trap-nights), and 32 rats (all Black Rats) in the non-treatment plot (12.48 rats per 100 corrected trap-nights). Two weeks after bait application, 0 rats were captured in the treatment plot and 47 rats (46 Black Rats and 1 Polynesian Rat) in the non-treatment plot (17.87 rats per 100 trap-nights) (Table 10). The post-treatment rat capture rate in the treatment plot was significantly lower than expected ($\chi^2 = 24.02$, df = 1, $P < 0.001$). Seven weeks after bait application, however, 1 rat (a Polynesian Rat) was caught in the treatment plot (0.32 rats per 100 trap-nights), and 27 rats (all Black Rats) were caught in the non-treatment plot (9.75 rats per 100 trap-nights) (Fig. 5). It was not until 21 weeks after the initial bait application (i.e., in June 2000) that the rat capture rate in the treatment plot increased to more than 50% of the pre-treatment capture rate (Fig. 5).

Three weeks after the second series of bait applications in July 2000, rat captures in the treatment plot declined from 17 Black Rats (6.34 rats per 100 trap-nights) to 1 Black Rat (0.34 rats per 100 trap-nights). However, rat captures also declined in the non-treatment plot, from 29 Black Rats (9.62 rats per 100 trap-nights) to 9 Black Rats (2.98 rats per 100 trap-nights) (Table 10, Fig. 5). The decline in the treatment plot relative to the non-treatment plot was 83% (Table 10). This was not statistically significant ($\chi^2 = 2.05$, df = 1, $P = 0.152$).

Three weeks after the third series of bait applications in October 2000, rat captures declined from 19 rats (all Black Rats) to 1 rat (a Black Rat) in the treatment plot and from 25 rats (23 Black Rats, 1 Polynesian Rat, and 1 Norway Rat) to 21 rats (19 Black Rats and 2 Polynesian Rats) in the non-treatment plot (Table 10, Fig. 5). The decline in the treatment plot relative to the non-treatment plot was 95% (Table 10). This is statistically significant ($\chi^2 = 7.38$, df = 1, $P = 0.007$).

The species, sex, and age composition of the rats caught in the treatment plot 2–3 weeks after bait application could not be compared to that caught before bait application because only two rats were caught, both male adult Black Rats, one after the July bait application and one after the October bait application (Table 11 and 12). The species, sex, and age composition of the rats caught in the non-treatment plot was similar before and 2–3 weeks after bait application in the treatment plot. Most rats caught were male adult Black Rats (Table 11 and 12).

Neither of the two rats captured in the treatment plot 2–3 weeks after bait application was a recapture (i.e., they had not been ear-tagged before treatment), whereas, on average, 22% of the rats captured in the non-treatment plot were recaptures (Table 13).

After the third series of bait applications, the rat capture rate in the treatment plot did not increase above 4 rats per 100 corrected trap-nights (less than half the initial capture rate), and further bait applications were not made. Live-trapping was not carried out in the non-treatment plot after November 2000, except once in April 2001, when the capture rate was 15.9 rats per 100 corrected trap-nights (Fig. 5).

Non-toxic census bait blocks. Before the initial bait application in January 2000, rats interfered with 5.6% of the census bait blocks in the treatment plot (no blocks missing) and 56.9% in the non-treatment plot (41.7% gnawed by rats plus 15.3% of blocks missing, presumably taken by rats) (Table 14, Fig. 6). Three weeks after the initial bait application, rat interference had declined to 0% in the treatment plot (100% reduction), but increased to 62.5% in the non-treatment plot (37.5% gnawed by rats, 25.0% missing). The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was statistically significant ($\chi^2 = 36.56$, df = 1, $P < 0.001$).

Repeat bait applications in July 2000 reduced the percentage of census blocks in the treatment plot interfered with by rats from 2.8% to 1.4% (no blocks missing before or after treatment), and in October 2000 from 34.7% (20.8% gnawed by rats, 13.9% missing) to 12.5% (4.2% gnawed by rats, 8.3% missing) (Table 14, Fig. 6). At the same time, the percentage of census blocks in the non-treatment plot interfered with by rats decreased from 73.6% (27.8% gnawed by rats, 45.8% missing) to 66.7% (23.6% gnawed by rats, 43.1% missing) in July 2000, and increased from 77.8% (11.1% gnawed by rats, 66.7% missing) to 88.9% (22.2% gnawed by rats, 66.7% missing) in October 2000. The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was, therefore, 44.8% in July and 68.5% in October 2000 (Table 14).

E. MOUSE ACTIVITY INDICES

WET FOREST

Live-trapping. Too few mice were caught to warrant rigorous examination of the data (Table 15). However, the average reduction in capture rates in the treatment plot, relative to the non-treatment plot, was 75.7%.

Non-toxic census bait blocks. There was insufficient interference by mice to census bait blocks to warrant rigorous examination of the data (Table 16). However, the average reduction in mouse interference in the treatment plot, relative to the non-treatment plot, was 69.2%.

Kill-trapping. No mice were caught in either the treatment or non-treatment plots before or after the first five bait application series. As a consequence, no further kill-trapping was carried out (see Methods).

MESIC FOREST

Live-trapping. The reduction in mouse capture rates in the treatment plot, relative to the non-treatment plot, was 100% after the first bait application series in January 2000, 95.5% after the second bait application series in July 2000, and 94.6% after the third bait application series in October 2000 (Table 17).

Non-toxic census bait blocks. There were not enough census bait blocks gnawed by mice in the treatment plot to warrant rigorous examination of the data (Table 18). However, there was no mouse gnawing on census bait blocks in the treatment plot after any of the bait application series.

Kill-trapping. No mice were caught in the treatment plot before or after the first bait application series in January 2000, and only one was caught in 83.5 trap-nights in the treatment plot before the second bait application series in July 2000. As a consequence of this, and the capture of several Red-billed Leiothrix and Northern Cardinals, no further kill-trapping was carried out (see Methods).

F. NON-TARGET SPECIES ASSESSMENT

No dead non-target species were found during any of the ground searches (about 4 person-hours, covering 10% of each plot) made after each bait application series in the wet forest and mesic forest (Appendix 20). However, some dead rats and dead non-target species were found during extensive visits to the plots for live-trapping, radio-telemetry, and census bait block surveys (at least 60 person-hours after each bait application) (see below).

Rats. In total, after all bait applications, 11 rats without radio transmitters were found dead in the wet forest treatment plot, and 4 rats without radio transmitters were found dead in the mesic forest treatment plot. Twelve of the 15 dead rats were Black Rats, but three could not be identified because they were too decomposed. The average diphacinone concentration in the livers of those found less than 2 weeks after bait application was 4.3 ppm ($n = 5$; rat # 4007, 4041, 4042, 4046, and 4143 in Appendix 19), those found 2–3 weeks after bait application was

3.4 ppm (n = 1; rat # 4047 in Appendix 19), and those found 6–7 weeks after bait application was 1.7 ppm (n = 2; rat # 4000 and 4040 in Appendix 19). Seven others were found more than 8 weeks after bait application and/or were too decomposed to analyze. Most of the rats that were found dead were found in the open, exposed to avian predators. However, based on the evidence from radio-collared rats (below), most of the rats that died would not have been found, because they died under cover, not in exposed places.

Of the five radio-collared rats recovered dead in the wet forest treatment plot, two were found in the center of hollow tree fern trunks and one in a fallen ohia tree trunk) inaccessible to potential avian predators, and three were found on the ground (two either partially or completed covered with fern fronds, and one on open ground exposed to potential avian predators) (Appendix 24). Of the six dead or dying radio-collared rats recovered in the mesic forest treatment plot, one was partially eaten by a mammalian predator within the center of a hollow trunk of a fallen koa, two were in underground burrows, two were lying on the surface of the forest floor exposed to view from above, not covered in vegetation, and one was barely alive within vegetation on the surface of the forest floor (this rat was euthanased with carbon dioxide). Of the remaining 11 dead radio-collared rats in the treatment plot that were unable to be recovered, six were located within the canopy of soapberry and koa trees and five were located underground in spaces between lava rocks. Thus, of the 22 radio-collared rats that died (five in the wet forest and 17 in the mesic forest treatment plots), three (13.6%) were in places exposed to avian predators.

House Mice. Two House Mice were found dead on the forest floor, exposed to avian predators, in the wet forest treatment plot on 13 September 2001, 3 weeks after bait application. Their livers contained 2.39 and 1.75 ppm diphacinone, respectively (mouse # 36 and 37 in Appendix 16).

Indian Mongooses. A male juvenile Indian Mongoose was found dead in the mesic forest treatment plot on 19 July 2000 (2 weeks after bait application). A necropsy revealed hemorrhaging typical of diphacinone poisoning. The liver contained 1.35 ppm diphacinone (sample # 4044 in Appendix 19). Green-dyed Indian Mongoose feces were found in the mesic forest treatment plot on 11 February 2000 (2 weeks after bait application).

Feral Cats. No Feral Cats were found dead. However, green-dyed Feral Cat feces were found in the wet forest treatment plot on 29 December 1999 (21 days after bait application) and 11 and 22 February 2000 (3 and 14 days after bait application).

Birds. No birds were found dead. However, an adult female Kalij Pheasant in a family group with two juveniles was observed pecking Ramik® Green bait in the mesic forest treatment plot on 6 July 2000 (1 day after bait application). Green-colored Kalij Pheasant feces were found in the wet forest treatment plot on 2 May 2000 (3 weeks after bait application) and 6 September 2001 (1 week after bait application).

Residues of diphacinone were detected in the livers of one of five Kalij Pheasants (range 0–0.09 ppm), three of six Red-billed Leothrix (0–0.70 ppm), and one of six Northern Cardinals (0–0.39 ppm) captured alive in the treatment plots 3–6 weeks after bait application (Table 19, Appendix 25). No diphacinone residues were detected in any of the four Japanese White-eyes. Because sample sizes were so small, results for the wet forest and mesic forest were combined. Even then, the sample sizes were too small to be certain of the true proportion of the bird

populations that had ingested diphacinone, and too small to say that no Japanese White-eyes had ingested diphacinone.

Kalij Pheasants pecked at less than 1% of the non-toxic census bait blocks in the first five monitoring sessions in the wet forest, and generally less than 20% thereafter, so rigorous examination of the data was not possible (Table 20). They also pecked at less than 20% of the census bait blocks in the mesic forest (Table 21). Over all bait application series, Kalij Pheasant pecking of census bait blocks increased from pre- to post-treatment in both the treatment and non-treatment plots, in both the wet forest (Table 20) and mesic forest (Table 21).

One or two Hawaiian Hawks were recorded in both the wet and mesic forest treatment plots throughout the study.

G. ENVIRONMENTAL CONDITIONS

WET FOREST

The mean daily rainfall in the treatment plot was less than 5 mm in the 2 weeks after all bait applications except in October 1999, December 1999, and November 2000 (Table 22). In October 1999, 51 mm of rain fell on day 14. In December 1999, 174 mm of rain fell on day 2 and 280 mm on day 3. In November 2000, 49 mm of rain fell on day 2, 17 mm on day 4, and 49 mm on day 5. Maximum temperatures ranged from 17.5 to 22.9°C, and minimum temperatures from 7.4 to 14.3°C.

MESIC FOREST

Little rain fell in the treatment plot in the first 2 weeks following the first two bait applications, in January and July 2000 (Table 23). However, 24 mm of rain fell on day 2, and it is estimated that more than 50 mm fell on day 7 and again on day 8, after the October 2000 bait application. Maximum temperatures ranged from 23.1 to 26.4°C, and minimum temperatures from 6.3 to 12.7°C.

V. DISCUSSION

The results from this study demonstrate that Ramik® Green bait containing 50 ppm (0.005%) diphacinone, hand-broadcast at 22.4 kg/ha, in two applications of 11.2 kg/ha, 4–6 days apart, is effective in reducing populations of rats (predominantly Black Rats) in both wet and mesic forest habitat in Hawaii. A reduction in rat populations of 100% in both forest types was measured by radio-telemetry. Live-trapping and non-toxic census bait blocks could not accurately measure efficacy, because they also measured immigration/reinvasion after bait application, but they indicated a reduction in rat numbers of 98–100% in the 2–4 weeks after the initial bait applications. The synchronous death of rats following the application of toxic baits, together with the presence of bait in their stomachs, signs of anticoagulant poisoning, and diphacinone residues in rat carcasses, indicate that the rats likely died of diphacinone poisoning. However,

the rat populations usually recovered within 2–5 months of bait application, presumably by reinvasion from surrounding areas.

Subsequent, repeat, hand-broadcast applications of bait were also highly successful in reducing rat populations, generally by 70–100% in the 2–4 weeks after bait application, as measured by live-trapping and census bait blocks, although there was some discrepancy between the two methods. For example, in the wet forest in June 2000, the efficacy was 29% as measured by live-trapping and 76% as measured by census bait blocks, and in August 2000 it was 70% as measured by live-trapping and 98% as measured by census bait blocks. It is difficult to know which of the two methods is more accurate, but the estimate of efficacy in June 2000 was low by both methods.

The different methods of monitoring the efficacy of toxic bait applications have different strengths and weaknesses. Radio-telemetry was the best method, because it enabled both the location and fate of known individuals to be determined. Radio-collared rats that lost their radio-transmitters, died, or moved out of the study plots could be excluded from the data analysis. However, radio-telemetry is costly and time-consuming, and was used to monitor only the first bait application in each treatment plot.

Live-trapping, with ear-tagging, was also useful but it was not possible to determine whether rats captured without ear-tags were present before bait application and survived (by not encountering baits, encountering baits but not eating them, or eating insufficient bait), or whether they had only moved into the plots after bait application. The small size of the study plots (200 m × 200 m), the ability of rats to move over distances of several hundred meters, and the length of time between bait application and live-trapping (up to 4 weeks), means that rats from surrounding areas could have moved into the plots in the interim, masking the true efficacy of the treatment. None of the rats ear-tagged in the treatment plots before treatment in either forest type were recaptured in the first 2–4 weeks after treatment, supporting the interpretation that those rats that were captured then were immigrants into the treatment plots rather than survivors of the treatment. One ear-tagged rat was recaptured in the treatment plot in the wet forest 9 weeks after treatment, and this may have been a survivor of the treatment, but equally may have moved out of the treatment plot before treatment, then moved back in again after treatment. This latter interpretation is plausible because four other rats moved at least 500 m between the treatment and non-treatment plots in 3–10 weeks. The use of radio-telemetry could have resolved this issue.

Gnawing on non-toxic census bait blocks was the most difficult method to interpret. In addition to the delay between bait application and post-treatment monitoring, allowing immigration/ reinvasion of rats to mask the true efficacy, as in live-trapping, other difficulties included (a) deciding which species (rats, mice, mongooses, birds, or invertebrates) had gnawed the census blocks, (b) interpreting the meaning of 100% of the census blocks being gnawed or removed by rats, and (c) deciding what to do about missing census blocks. For example, it was not always easy to determine which species had gnawed the census blocks, because gnawing by one species may have been masked by subsequent gnawing by another species. This may have resulted in an underestimate of some species. When 100% of the census blocks have been gnawed or removed by rats, the maximum size of the rat population cannot be estimated. Even when interference approaches 100%, rat density is likely to be underestimated. Percentage interference to census blocks should be log-transformed, because there is unlikely to be a linear

relationship between the level of interference and animal density (Caughley 1977; Spurr 1995). Limiting the period of monitoring to 1 day might help reduce the level of interference for the first monitoring session, but it does not help for subsequent monitoring sessions, because rats learn where the census blocks are and return to them rather than interfere with them at random. The technique also has limitations when census blocks go missing, because it is not possible to determine which species is responsible for them going missing. Missing census blocks can be deleted from the calculations, but this can exaggerate the percentage interference to the remaining blocks by less common species. For example, if 50% of the blocks are missing, and rats gnawed 90% and mice 10% of the remaining blocks, and rats were responsible for removing the missing blocks, then the rat population will have been underestimated and the mouse population overestimated. Really, in this example, rats interfered with 95% of the blocks and mice 5%. When 100% of the blocks go missing, the technique simply does not work. Nevertheless, when census blocks remain in the treatment plot after application of toxic baits, and no (or little) gnawing is observed, this indicates a successful reduction in rat numbers.

The age of the bait used does not appear to have affected efficacy. The bait used in the wet forest in June 2000 was 12 months old and in August 2000 it was 15 months old. If this bait had a low palatability to rats and/or a low concentration of diphacinone (which was not determined), it could have allowed rats to survive. However, the bait disappeared rapidly from the forest floor (presumably eaten by rats) in both June and August, indicating that it was still palatable to rats. Also, bait from the same lot number used in the mesic forest in October 2000 (when 16 months old) killed 95% of the rats as measured by live-trapping and 68.5% of the rats as measured by census bait blocks. Bait from a different lot number used in the wet forest in December 2001 when 15 months old, and with a diphacinone concentration of only 40.3 ppm, killed 91% of the rats.

The higher proportion of Norway Rats captured in the treatment plot in the wet forest 2–4 weeks after bait application (compared to before) indicates either that Norway Rats survived better or reinvaded more quickly than Black or Polynesian Rats. Alternatively, the result is a chance result. This needs further investigation. Whichever explanation is correct, the species composition 2–3 months after bait application was similar to that before bait application.

House Mouse numbers were also reduced by the broadcast application of 50 ppm diphacinone bait, but not by as much as rat numbers. The LD_{50} of diphacinone for mice (50–300 mg/kg) is higher than for rats (0.3–7 mg/kg), and mice have smaller home ranges than rats. These aspects need further investigation.

The discovery of a mongoose carcass with typical signs of anticoagulant poisoning and diphacinone residues in its liver, and the discovery of green-dyed mongoose and cat feces, indicates that these species also may be affected by the broadcast application of 50 ppm diphacinone bait for rat control. The affected animals may have eaten Ramik® Green bait directly or eaten rats that had eaten bait. In a recent field trial, application of 50 ppm diphacinone bait blocks (J.T. Eaton Corporation) in bait stations was highly effective in reducing mongoose numbers (Smith et al. 2000). Radio-telemetry and live-trapping will be needed in future studies to determine the impacts of broadcast application of Ramik® Green bait for rat control on mongoose numbers.

Bait remained on the forest floor, potentially available to non-target species, for at least 7 and up to more than 24 days after application. The variable length of time that the bait remained on the forest floor may have been related to a combination of factors, including rainfall and rat density. The length of time that bait remained on the forest floor has not appeared to present a hazard to birds because no bird mortality was observed. A Kalij Pheasant was observed eating Ramik® Green bait, and green dye from Ramik® Green bait was observed in Kalij Pheasant feces. Diphacinone residues were found in the livers of Kalij Pheasants and also in Red-billed Leothrix and Northern Cardinals (all introduced seed-eating and/or omnivorous birds), implying they had eaten baits. However, the concentration of diphacinone (up to 0.7 ppm) found in these birds is much less than the LD_{50} of diphacinone for the Bobwhite Quail (1630 ppm) and Mallard Duck (3158 ppm). Red-billed Leothrix, Japanese Bush Warbler (*Cettia diphone*), and Erckel's Francolin (*Francolinus erckelli*) have been reported eating placebo Ramik® Green baits placed on the forest floor (P. Dunlevy and E. Campbell pers. comm.).

Based on the success of this and previous studies (Lindsey and Mosher 1994; Swift 1998; Dunlevy et al. 2000) suggesting high efficacy against rats and minimal hazards to non-target species from 50 ppm diphacinone baits, we recommend that an aerial-broadcast application of Ramik® Green baits be made in a conservation area in Hawaii to evaluate the effectiveness and safety of this technique for the control of rat populations.

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VII. LITERATURE CITED

Atkinson, I. A. E. 1977. A reassessment of factors, particularly *Rattus rattus* L., that influenced the decline of endemic forest birds in the Hawaiian islands. *Pacific Science 31:* 109–133.

Atkinson, I. A. E. 1985. The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifauna. *In*: Moors, P. J. (Ed.), *Conservation of island birds*. International Council for Bird Preservation, Technical Bulletin 3. Cambridge, England, pp. 35–81.

Baker, J. K.; Allen, M. S. 1978. Roof rat depredations on *Hibiscadelphus* (Malvaceae) trees. *In*: Smith, C. W. (Ed.), Proceedings of the 2nd Hawaii Volcanoes National Park Natural Sciences Conference, Honolulu, pp. 2–5.

Baldwin, P. H. 1945. The Laysan Rail. *Audubon 47*: 383–348.

Beccari, O.; Rock, J. F. 1921. A monographic study of the genus *Pritchardia*. Memoirs of the B. P. Bishop Museum Vol. 8, No. 1. Bishop Museum Press, Honolulu.

Berger, A. J. 1981. Hawaiian birdlife, 2nd ed. University of Hawaii Press, Honolulu.

Buckle, A. P.; Fenn, M. G. P. 1992. Rodent control in the conservation of endangered species. *In*: Borrecco, J. E.; Marsh, R. E. (Eds), Proceedings of the 15th Vertebrate Pest Conference. University of California, Davis, U.S.A., pp. 36–51.

Caughley, G.C. 1977. Analysis of vertebrate populations. John Wiley & Sons, London, U.K. 234 pp.

Cox, P.; Smith, R. H. 1992. Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behaviour. *In*: Borrecco, J. E.; Marsh, R. E. (Eds), Proceedings of the 15th Vertebrate Pest Conference. University of California, Davis, U.S.A., pp. 165–170.

Cuddihy, L. W.; Stone, C. P. 1990. Alteration of native Hawaiian vegetation. Cooperative National Park Resources Studies Unit, University of Hawaii. University of Hawaii Press, Honolulu.

Dunlevy, P. A.; Campbell III, E. Wm.; Lindsey, G. D. 2000. Broadcast application of a placebo rodenticide bait in a native Hawaiian forest. *International Biodeterioration & Biodegradation 45*: 199–208.

Ebenhard, J. 1988. Introduced birds and mammals and their ecological effects. *Swedish Wildlife Research 13*: 5–107.

Empson, R. A.; Miskelly, C. M. 1999. The risks, costs and benefits of using brodifacoum to eradicate rats from Kapiti Island, New Zealand. *New Zealand Journal of Ecology 23*: 241–254.

Extoxnet. 1993. Diphacinone. Cooperative Extension Office, Cornell University.

Godfrey, M. E. R. 1985. Non-target and secondary poisoning hazards of "second generation" anticoagulants. *Acta Zoologica Fennica 173*: 209–212.

Hadfield, M. G.; Miller, S. E.; Carwile, A. H. 1993. The decimation of endemic Hawaiian tree snails by alien predators. *American Zoologist 33:* 610–622.

Hooker, S.; Innes, J. 1995. Ranging behaviour of forest-dwelling ship rats, *Rattus rattus*, and effects of poisoning with brodifacoum. *New Zealand Journal of Zoology 22*: 291–304.

Innes, J.; Warburton, B.; Williams, D.; Speed, H.; Bradfield, P. 1995. Large-scale poisoning of ship rats (*Rattus rattus*) in indigenous forests of the North Island, New Zealand. *New Zealand Journal of Ecology 19:* 5–17.

Innes, J.; Hay, R.; Flux, I.; Bradfield, P.; Speed, H.; Jansen, P. 1999. Successful recovery of North Island kokako *Callaeas cinerea wilsoni* populations, by adaptive management. *Biological Conservation 87*: 201–214.

IPCS. 1995. Environmental Health Criteria 175: Anticoagulant rodenticides. World Health Organization, Geneva.

IUCN 2002. 2002 IUCN Red List of Threatened Species™. International Union for Conservation of Nature and Natural Resources, Species Survival Commission, Gland, Switzerland.

Johnson, M. S. 1945. Rodent control on Midway Islands. *U. S. Naval Med. Bull. 45*: 384–398.

Keith J. O.; Hirata, D. N.; Espy, D. L.; Greiner, S.; Griffin, D. 1990. Field evaluation of 0.00025% diphacinone bait for mongoose control in Hawaii. Denver Wildlife Research Center, Denver CO. 52 pp.

Kepler, C. B. 1967. Polynesian rat predation on nesting Laysan albatrosses and other Pacific seabirds. *Auk 84*: 426–430.

Lindsey, G. D.; Forbes, C. 2000. Effectiveness of hand broadcast baiting with 0.005% diphacinone bait pellets in reducing rat populations in Hawaiian forests. USGS, Biological Resources Division, Hawaii Volcanoes National Park, Hawaii (unpublished report). 23 pp.

Lindsey, G. D.; Mosher, S. M. 1994. Tests indicate minimal hazard to 'io from diphacinone baiting. *Hawaii's Forests and Wildlife 4*: 1,3.

Lindsey, G. D.; Mosher, S. M.; Fancy, S. G.; Smucker, T. D. 1999. Population structure and movements of introduced rats in an Hawaiian rainforest. *Pacific Conservation Biology 5*: 94–102.

McCullagh, P.; Nelder, J.A. 1989. Generalized linear models. Chapman and Hall, London, U.K.

Mendenhall, V. M.; Pank, L. F. 1980. Secondary poisoning of owls by anticoagulant rodenticides. *Wildlife Society Bulletin 8*: 311–315.

Moors, P. J.; Atkinson, I. A. E.; Sherley, G. H. 1992. Reducing the rat threat to island birds. *Bird Conservation International 2:* 93–114.

Mueller-Dombois, D.; Lamoureux, C. H. 1967. Soil-vegetation relationships in Hawaiian Kipukas. *Pacific Science 21*: 286–299.

Nelson, J. T.; Woodworth, B. L.; Fancy, S. J.; Lindsey, G. D.; Tweed, E. J. 2002. Effectiveness of rodent control and monitoring techniques for a montane rainforest. *Wildlife Society Bulletin 30*: 82–92.

Nelson, L. Jr.; Clark, F.W. 1973. Correction for sprung traps in catch/effort calculations of trapping results. *Journal of Mammalogy 54:* 295–298.

Perkins, R. C. L. 1903. Vertebrata. *In*: Sharp, D. (Ed.), Fauna Hawaiiensis, Vol I, pt. IV. The University Press, Cambridge, England.

Richardson, F.; Woodside; D. H. 1954. Rediscovery of the nesting of the Dark-rumped Petrel in the Hawaiian Islands. *Condor 56*: 323–327.

Russell, C. A. 1980. Food habits of the roof rat (*Rattus rattus*) in two areas of Hawaii Volcanoes National Park. *In*: Smith, C. W. (Ed.), Proc. 3rd Conference in Natural Sciences, Hawaii Volcanoes National Park. Honolulu, U.S.A., pp. 269–272.

Savarie, P. J.; Hayes, D. J.; McBrice, R. T.; Roberts, J. D. 1979. Efficacy and safety of diphacinone as a predacide. Avian and Mammalian Wildlife Toxicology, ASTM STP 693. Kenaga, E. E. (Ed.), American Society for Testing and Materials, pp. 69–79.

Scott, J. M.; Mountainspring, S.; Ramsey, F. L.; Kepler, C. B. 1986. Forest bird communities of the Hawaiian Islands: their dynamics, ecology, and conservation. *Studies in Avian Biology 9*: 1–431.

Scowcroft, P. G.; Sakai; H. F. 1984. Stripping of *Acacia koa* bark by rats on Hawaii and Maui. *Pacific Science 38*: 80–86.

Seto, N. W. H.; Conant, S. 1996. The effects of rat (*Rattus rattus*) predation on the reproductive success of the Bonin Petrel (*Pterodroma hypoleuca*) on Midway Atoll. *Colonial Waterbirds 9*: 171–322.

Smith, D. G.; Polhemus, J. T.; Vander Werf, E. A. 2000. Efficacy of fish-flavored diphacinone bait blocks for controlling small Indian mongoose (*Herpestes auropunctatus*) populations in Hawai'i. *'Elepaio 60*: 47–51.

Spurr, E. B. 1995. Evaluation of non-toxic bait interference for indexing brushtail possum density. *New Zealand Journal of Ecology 19*: 123–130.

Snetsinger, T. J.; Fancy, S. G.; Simon, J. C.; Jacobi, J. D. 1994. Diets of owls and feral cats in Hawaii. *'Elepaio 54*: 47–50.

Stone, C. P. 1985. Alien animals in Hawaii's native ecosystems: toward controlling the adverse effects of introduced vertebrates. *In*: Stone, C. P.; Scott, J. M. (Eds), Co-operative National Park Resources Studies Unit. University of Hawaii, Honolulu, U.S.A., pp. 251–297.

Stone, C.P.; Pratt, L.W. 2002. Hawai'i's plants and animals: Biological sketches of Hawai'i Volcanoes National Park. 2nd ed. Hawaii Natural History Association, Hawaii Volcanoes National Park. 408 pp.

Stone, C.P.; Scott, J.M. 1985. Hawai'i's terrestrial ecosystems: Preservation and management. Cooperative National Park Resources Studies Unit, University of Hawaii, Honolulu. 584 pp.

Stone, C. P.; Dusek, M.; Aeder, M. 1995. Use of an anticoagulant to control mongooses in nene breeding habitat. *'Elepaio 54*: 73–78.

Sugihara, R. T. 1997. Abundance and diets of rats in two native Hawaiian forests. *Pacific Science 51*: 189–198.

Swift, C. E. 1998. Laboratory bioassays with wild-caught black (*Rattus rattus*) and Polynesian (*R. exulans*) rats to determine minimum amounts of Ramik Green (0.005% diphacinone) and exposure times for field broadcast applications in Hawaii. MSc. Thesis, University of Hawaii-Manoa, Honolulu, Hawaii. 92 pp.

Tomich, P. Q. 1986. Mammals in Hawaii. 2nd ed. Bishop Museum Press, Honolulu, Hawaii, USA. 375 pp.

Towns, D.; McFadden, I.; Lovegrove, T. 1993. Off-shore islands co-operative conservation project with ICI Crop Care Division: Phase one (Stanley Island). Science and Research Internal Report No. 138. Department of Conservation, Wellington, New Zealand. 24 pp.

Towns, D.; McFadden, I.; Thomson, P.; Robertson, H.; Colbourne, R. 1994. Off-shore islands co-operative conservation project with ICI Crop Care Division: Phase two (Red Mercury Island). Science and Research Internal Report No. 142. Department of Conservation, Wellington, New Zealand. 12 pp.

Towns, D.; McFadden, I.; Thomson, P. 1995. Off-shore islands co-operative conservation project with ICI Crop Care Division: Phase three (Cuvier Island). Science and Research Internal Report No. 150. Department of Conservation, Wellington, New Zealand. 13 pp.

U.S. Fish and Wildlife Service 1996. Recovery plan for the Big Island plant cluster. U.S. Fish and Wildlife Service, Portland, Oregon.

U.S. Fish and Wildlife Service 2002. Endangered and threatened wildlife and plants; designations of critical habitat for plant species from the island of Hawaii, Hawaii. *Federal Register 67(102)*: 36967-37016.

Van Riper, C. III. 1978. The breeding ecology of the amakihi (*Loxops virens*) and palila (*Psittirostra bailleui*) on Mauna Kea, Hawaii. Unpublished PhD thesis, University of Hawaii, Hawaii. 166 pp.

Van Riper, C. III. 1980. Observations on the breeding of the Palila *Psittirostra bailleui* of Hawaii. *Ibis 122*: 462–475.

Woodward, P. W. 1972. The natural history of Kure Atoll, Northwestern Hawaiian Islands. *Atoll Research Bulletin 164*.

VIII. TABLES

Table 1. Physical characteristics of Ramik® Green baits used for the hand-broadcast trials, Hawaii Volcanoes National Park, measured in September 1999, October 1999, and May 2001 (after being stored at ambient temperature in a dehumidified room).

Mean ± standard error	Lot No. 125218 (manufactured May 1999)	Lot No. 144548 (manufactured September 2000)
September 1999 (n=205)		
Weight (g)	5.8 ± 0.05	
Length (mm)	22.0 ± 0.16	
Diameter (mm)	20.2 ± 0.06	
October 1999 (n=205)		
Weight (g)	5.8 ± 0.05	
Length (mm)	22.1 ± 0.17	
Diameter (mm)	20.3 ± 0.05	
May 2001 (n=24)		
Weight (g)	6.2 ± 0.10	6.5 ± 0.1
Length (mm)	22.7 ± 0.30	23.5 ± 0.4
Diameter (mm)	20.9 ± 0.20	20.5 ± 0.4

Table 2. Diphacinone content (mean ± standard error) of Ramik® Green baits used in hand-broadcast trials, Hawaii Volcanoes National Park, measured at various times after manufacture. (Lot No. 125218 manufactured May 1999, Lot No. 144548 manufactured September 2000).

Lot No.	Date of analysis (month, year)	Age of baits (months)	Diphacinone content (ppm)	Analytical laboratory
125218	May 1999	0	51	HACCO
	April 2000	12	46.4 ± 0.6	Genesis
144548	September 2000	0	51	HACCO
	January 2002	16	40.3 ± 0.9	Genesis
	April 2002	19	39.4 ± 0.7	Genesis
	May 2002	20	44	HACCO

See Appendix 15 and 16 for details.

Table 3. Number of radio-collared rats alive, and percent change in the number of radio-collared rats alive in the treatment plot relative to the non-treatment plot, after hand-broadcast application of Ramik® Green bait in the wet forest, Hawaii Volcanoes National Park, 7 and 12 October 1999.

Date	Non-treatment plot	Treatment plot	% change after treatment
Before (29 Sep–1 Oct 99)	11	5	
After (19 Oct–22 Oct 99)	9	0	–100

Table 4. Rat live captures per 100 corrected trap-nights in treatment and non-treatment plots 1–2 weeks before and 2–4 weeks after repeat hand-broadcast applications of Ramik® Green bait in wet forest, Hawaii Volcanoes National Park.
(See Methods for calculation of % kill, Chi-square, and *P* values).

Date start bait application	Non-treatment plot			Treatment plot			% kill in treatment plot	Chi-square value	*P* value
	Before	After	% change	Before	After	% change			
7 Oct 1999	5.58	8.47	+51.8	4.48	0	–100	100	6.64	0.010
8 Dec 1999	4.07	4.29	+5.3	7.10	0	–100	100	6.49	0.011
8 Feb 2000	4.20	6.59	+56.9	5.58	0	–100	100	7.61	0.006
12 Apr 2000	3.69	7.36	+99.3	7.99	0	–100	100	10.86	0.001
14 Jun 2000	9.81	8.06	–17.8	7.83	4.59	–41.4	29	0.14	0.706
31 Aug 2000	5.41	9.93	+83.7	4.62	2.55	–44.9	70	1.65	0.199
9 Nov 2000	12.82	4.20	–67.2	18.61	0	–100	100	6.55	0.010
18 Jan 2001	3.93	2.28	–41.9	10.40	0.32	–97.0	95	3.42	0.064
19 Mar 2001	5.04	3.45	–31.6	3.97	0.63	–84.2	77	1.07	0.301
24 May 2001	6.03	1.90	–68.5	13.41	0.74	–94.5	83	1.37	0.242
23 Aug 2001	4.18	5.01	+19.7	3.40	0	–100	100	3.87	0.049
17 Dec 2001	12.50	6.95	–44.4	7.45	0.64	–91.5	85	2.45	0.117

Table 5. Species composition of rats (%) caught in the treatment and non-treatment plots 1–2 weeks before and 2–4 weeks after hand-broadcast applications of Ramik® Green bait in wet forest, Hawaii Volcanoes National Park.
(All applications combined).

Species	Non-treatment plot		Treatment plot	
	Before	After	Before	After
Black Rat	78.3	75.9	72.0	57.1
Polynesian Rat	12.3	16.6	20.8	21.4
Norway Rat	9.4	7.5	7.2	21.4
Total caught (n)	(212)	(199)	(250)	(28)

Table 6. Sex and age classes of rats (%) caught in the treatment and non-treatment plots 1–2 weeks before and 2–4 weeks after hand-broadcast applications of Ramik® Green bait in wet forest, Hawaii Volcanoes National Park.
(All applications combined. Sample sizes differ from Table 5 because some rats escaped before they could be sexed or aged).

Sex and age class	Non-treatment plot		Treatment plot	
	Before	After	Before	After
Adult male	28.8	31.3	44.4	42.3
Adult female	22.0	23.6	22.6	15.4
Adult sex unknown	7.8	3.3	2.5	0.0
Juvenile male	25.9	25.8	10.3	19.2
Juvenile female	10.2	10.4	14.8	11.5
Juvenile sex unknown	5.5	5.5	5.3	11.5
Total caught (n)	(205)	(182)	(243)	(26)

Table 7. Percentage of ear-tagged rats recaptured in treatment and non-treatment plots 2–4 weeks after repeat hand-broadcast applications of Ramik® Green bait in wet forest, Hawaii Volcanoes National Park.
(n = number of rats captured).

Date start bait application	Non-treatment plot		Treatment plot	
	%	n	%	n
7 Oct 1999	4.0	25	0	0
8 Dec 1999	30.8	13	0	0
8 Feb 2000	40.0	20	0	0
12 Apr 2000	27.3	22	0	0
14 Jun 2000	21.7	23	0	13
31 Aug 2000	10.3	29	0	8
9 Nov 2000	23.1	13	0	0
18 Jan 2001	42.9	7	0	1
19 Mar 2001	33.3	9*	0	2
24 May 2001	40.0	5	0	2
23 Aug 2001	0	13	0	0
17 Dec 2001	31.6	19	0	2
Average (total)	22.2	(198)	(0)	(28)

* Excludes one rat whose recapture status was not recorded.

Table 8. Percentage of census bait blocks gnawed or removed by rats in treatment and non-treatment plots 1–2 weeks before and 2–3 weeks after repeat hand-broadcast applications of Ramik® Green bait in wet forest, Hawaii Volcanoes National Park (See Methods for calculation of % kill, Chi-square, and *P* values).

Date start bait application	Non-treatment plot			Treatment plot			% kill in treatment plot	Chi-square value	*P* value
	Before	After	% change	Before	After	% change			
7 Oct 1999	36.1	87.5	+142.4	29.6	1.4	–95.3	98	378.45	<0.001
8 Dec 1999	95.8	98.6	+2.9	12.5	5.6	–55.2	57	15.75	<0.001
8 Feb 2000	97.2	100.0	+2.9	2.7	0	–100	100	19.43	<0.001
12 Apr 2000	100.0	97.2	–2.8	15.3	1.4	–90.8	91	4.02	0.045
14 Jun 2000	100.0	98.6	–1.4	23.6	5.6	–76.3	76	3.36	0.067
31 Aug 2000	93.1	100.0	+7.4	72.2	1.4	–98.1	98	204.67	<0.001
9 Nov 2000	98.6	100.0	+1.4	52.8	4.2	–92.0	92	23.55	<0.001
18 Jan 2001	100.0	100.0	0	–	0	–100	100	–	–
19 Mar 2001	100.0	100.0	0	9.9	0	–100	100	18.07	<0.001
24 May 2001	100.0	100.0	0	22.1	12.5	–43.4	43	0.91	0.340
23 Aug 2001	100.0	100.0	0	54.2	5.6	–89.7	90	13.71	<0.001
17 Dec 2001	93.0	100.0	+7.5	75.0	4.2	–94.4	95	183.24	<0.001

Note: Data from the before 18 January 2001 bait application in the treatment plot were lost (see Appendix 3, deviation 2).

Table 9. Number of radio-collared rats alive, and percent change in the number of radio-collared rats alive in the treatment plot relative to the non-treatment plot, after hand-broadcast application of Ramik® Green bait in the mesic forest, Hawaii Volcanoes National Park, 27 January and 1 February 2000.

Date	Non-treatment plot	Treatment plot	% change after treatment
Before (19 Jan–22 Jan 00)	13	17	
After (8 Feb–11 Feb 00)	12	0	–100

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Table 10. Rat live captures per 100 corrected trap-nights in treatment and non-treatment plots 1 week before and 2–3 weeks after repeat hand-broadcast applications of Ramik® Green bait in mesic forest, Hawaii Volcanoes National Park. (See Methods for calculation of % kill, Chi-square, and *P* values).

Date start bait application	Non-treatment plot			Treatment plot			% kill in treatment plot	Chi-square value	*P* value
	Before	After	% change	Before	After	% change			
27 Jan 2000	12.48	17.87	+43.2	10.86	0	–100	100	24.02	<0.001
5 Jul 2000	9.62	2.98	–69.9	6.34	0.34	–94.6	82.7	2.05	0.152
25 Oct 2000	8.67	7.39	–14.8	6.83	0.32	–95.3	94.5	7.38	0.007

Table 11. Species composition of rats (%) caught in treatment and non-treatment plots 1 week before and 2–3 weeks after hand-broadcast applications of Ramik® Green bait in mesic forest, Hawaii Volcanoes National Park.
(All applications combined).

Species	Non-treatment plot		Treatment plot	
	Before	After	Before	After
Black Rat	97.7	96.1	96.9	100
Polynesian Rat	1.1	1.3	3.1	0
Norway Rat	1.1	2.6	0.0	0
Total caught (n)	(86)	(77)	(65)	(2)

Table 12. Sex and age class of rats (%) caught in treatment and non-treatment plots 1 week before and 2–3 weeks after hand-broadcast applications of Ramik® Green bait in mesic forest, Hawaii Volcanoes National Park.
(All applications combined. Sample sizes differ from Table 11 because some rats escaped before they could be sexed or aged).

Sex and age class	Non-treatment plot		Treatment plot	
	Before	After	Before	After
Adult male	30.5	30.6	51.9	100
Adult female	28.0	34.7	30.8	0
Adult sex unknown	11.0	2.8	0.0	0
Juvenile male	12.2	22.2	5.8	0
Juvenile female	17.1	6.9	11.5	0
Juvenile sex unknown	1.2	2.8	0.0	0
Total caught (n)	(82)	(72)	(52)	(2)

Table 13. Percentage of ear-tagged rats recaptured in treatment and non-treatment plots 2–3 weeks after repeat hand-broadcast applications of Ramik® Green bait in mesic forest, Hawaii Volcanoes National Park.
(n = number of rats captured).

Date start bait application	Non-treatment plot		Treatment plot	
	%	n	%	n
27 Jan 2000	19.1	47	0	0
5 Jul 2000	11.1	9	0	1
25 Oct 2000	33.3	21	0	1
Average (total)	22.1	(77)	(0)	(2)

Table 14. Percentage of census bait blocks gnawed or removed by rats in treatment and non-treatment plots, 1–2 weeks before and 2–3 weeks after repeat hand-broadcast applications of Ramik® Green bait in the mesic forest, Hawaii Volcanoes National Park. (See Methods for calculation of % kill, Chi-square, and *P* values).

Date start bait application	Non-treatment plot			Treatment plot			% kill in treatment plot	Chi-square value	*P* value
	Before	After	% change	Before	After	% change			
27 Jan 2000	56.9	62.5	+9.8	5.6	0	–100	100	36.56	<0.001
5 Jul 2000	73.6	66.7	–9.4	2.8	1.4	–50.0	44.8	0.52	0.469
25 Oct 2000	77.8	88.9	+14.3	34.7	12.5	–64.0	68.5	71.10	<0.001

Table 15. House Mouse live-captures per 100 corrected trap-nights 1–2 weeks before and 2–4 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in wet forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
7 Oct 1999	0	0.34	0	0.31
8 Dec 1999	0	0	4.97	0
8 Feb 2000	0.65	0.33	0.66	0
12 Apr 2000	0	0	0.69	0.32
14 Jun 2000	0	0.35	0.71	0.71
31 Aug 2000	0	0	0	0
9 Nov 2000	0	0	3.62	0
18 Jan 2001	0.33	0	0.35	0.63
19 Mar 2001	0	0	0.33	0
24 May 2001	1.13	1.14	0.81	0
23 Aug 2001	7.11	1.93	0	0.97
17 Dec 2001	2.94	6.22	1.77	0
Average	1.01	0.86	1.16	0.24

Table 16. Percentage of census bait blocks gnawed by House Mice 1–2 weeks before and 2–3 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in wet forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
7 Oct 1999	0	1.4	0	1.4
8 Dec 1999	0	0	0	1.4
8 Feb 2000	6.9	5.6	0	0
12 Apr 2000	0	5.6	0	0
14 Jun 2000	0	1.4	4.2	0
31 Aug 2000	0	0	0	0
9 Nov 2000	0	0	0	0
18 Jan 2001	-	-	-	-
19 Mar 2001	0	0	0	0
24 May 2001	0	0	1.5	0
23 Aug 2001	0	12.5	4.2	5.6
17 Dec 2001	2.8	0	0	0
Average	0.88	2.41	0.90	0.76

Note: Data from before 18 January 2001 bait application in the treatment plot were lost (see Appendix 3, deviation 2).

Table 17. House Mouse live-captures per 100 corrected trap-nights 1–2 weeks before and 2–3 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in mesic forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
27 Jan 2000	21.83	10.65	9.81	0
5 July 2000	0.66	0.66	7.46	0.34
25 Oct 2000	3.12	3.86	9.35	0.63
Average	8.53	5.04	8.87	0.32

Table 18. Percentage of census bait blocks gnawed by House Mice 1–2 weeks before and 2–3 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in mesic forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
27 Jan 2000	27.8	9.7	9.7	0
5 July 2000	6.9	0	0	0
25 Oct 2000	0	0	0	0
Average	11.6	3.2	3.2	0

Table 19. Diphacinone concentration (ppm) detected in the livers of non-target birds collected alive in the treatment plots 3–7 weeks after hand-broadcast application of Ramik® Green bait in wet forest and mesic forest, Hawaii Volcanoes National Park.
(Data from the two treatment plots combined).

Species	Number sampled	% with diphacinone	Diphacinone (ppm)	
			Mean	Range
Kalij Pheasant	5	20	0.02	0–0.09
Red-billed Leiothrix	6	50	0.28	0–0.70
Northern Cardinal	6	17	0.07	0–0.39
Japanese White-eye	10	0	0	0–0.00

Table 20. Percentage of census bait blocks pecked by Kalij Pheasants 1–2 weeks before and 2–4 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in the wet forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
7 Oct 1999	0	0	0	0
8 Dec 1999	0	0	0	0
8 Feb 2000	0	0	0	0
12 Apr 2000	0	0	0	0
14 Jun 2000	0	0	1.4	1.4
31 Aug 2000	0	0	15.3	2.8
9 Nov 2000	1.4	0	6.9	0
18 Jan 2001	-	-	-	-
19 Mar 2001	0	0	9.9	15.3
24 May 2001	0	0	16.2	38.9
23 Aug 2001	0	0	11.1	12.5
17 Dec 2001	6.9	0	25.0	12.5
Average	0.7	0.0	7.8	7.6

Note: Data from before 18 January 2001 bait application in the treatment plot were lost (see Appendix 3, deviation 2).

Table 21. Percentage of census bait blocks pecked by Kalij Pheasants 1–2 weeks before and 2–3 weeks after hand-broadcast application of Ramik® Green bait in the treatment plot in mesic forest, Hawaii Volcanoes National Park.

Date start bait application	Non-treatment plot		Treatment plot	
	Before	After	Before	After
27 Jan 2000	9.7	11.1	0	0
5 July 2000	2.8	18.1	5.6	8.5
25 Oct 2000	11.1	5.6	0	12.5
Average	7.9	11.6	1.9	7.0

Table 22. Daily mean rainfall, and maximum and minimum temperatures from the treatment plot in the wet forest for 14 days after hand-broadcast application of Ramik® Green bait (unless otherwise stated).

Bait application		Rainfall (mm)	Temperature (°C)	
No.	Start date	Mean	Maximum	Minimum
1	7 Oct 1999	5.9	21.9	12.3
2	8 Dec 1999	35.1	20.5	11.1
3	8 Feb 2000	0.2	22.9	7.4
4	12 Apr 2000	2.3	22.1	11.2
5	14 Jun 2000	4.2	20.4	13.5
6	31 Aug 2000 (15 days)	4.2	20.6	14.3
7	9 Nov 2000 (12 days)	10.3	18.6	13.5
8	18 Jan 2001	1.6	21.5	9.0
9	19 Mar 2001	1.5	19.1	10.4
10	24 May 2001	1.7	17.9	10.9
11	23 Aug 2001 (5 days)	4.4	18.0	11.3
12	17 Dec 2001 (16 days)	1.6	17.5	11.4

Table 23. Daily mean rainfall, and maximum and minimum temperatures from the treatment plot in the mesic forest for 14 days after hand-broadcast application of Ramik® Green bait (unless otherwise stated).

Bait application		Rainfall (mm)	Temperature (°C)	
No.	Start date	Mean	Maximum	Minimum
1	27 Jan 2000	0.1	23.1	6.3
2	5 Jul 2000	1.6	23.8	12.7
3	25 Oct 2000 (6 days)	6.1	26.4	12.4

IX. FIGURES

Figure 1. Disappearance of Ramik® Green bait (expressed as % baits remaining) in wet forest, Hawaii Volcanoes National Park. (dotted lines represent different hand-broadcast bait applications between October 1999 and December 2001, dark solid line represents log-smoothed average)

Figure 2. Disappearance of Ramik® Green bait (expressed as % baits remaining) in mesic forest, Hawaii Volcanoes National Park, after hand-broadcast bait applications in January, July, and October 2000

Figure 3. Live captures of rats (per 100 corrected trap-nights) in wet forest, Hawaii Volcanoes National Park (arrows indicate date of bait applications)

Figure 4. Percentage of non-toxic census bait blocks interfered with by rats in wet forest, Hawaii Volcanoes National Park (arrows indicate date of bait applications)

Figure 5. Live captures of rats (per 100 corrected trap-nights) in mesic forest, Hawaii Volcanoes National Park (arrows indicate date of bait applications)

Figure 6. Percentage of non-toxic census bait blocks interfered with by rats in mesic forest, Hawaii Volcanoes National Park (arrows indicate date of bait applications)

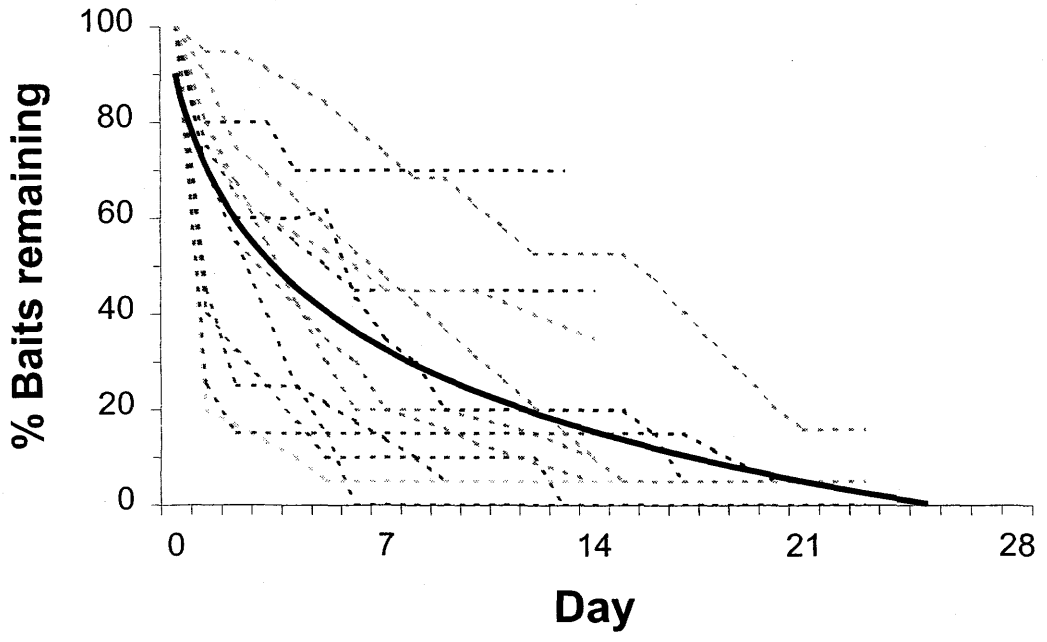

Figure 1. Disappearance of Ramik® Green bait (expressed as % baits remaining) in wet forest, Hawaii Volcanoes National Park (dotted lines represent different hand-broadcast bait applications between October 1999 and December 2001, dark solid line represents log-smoothed average).

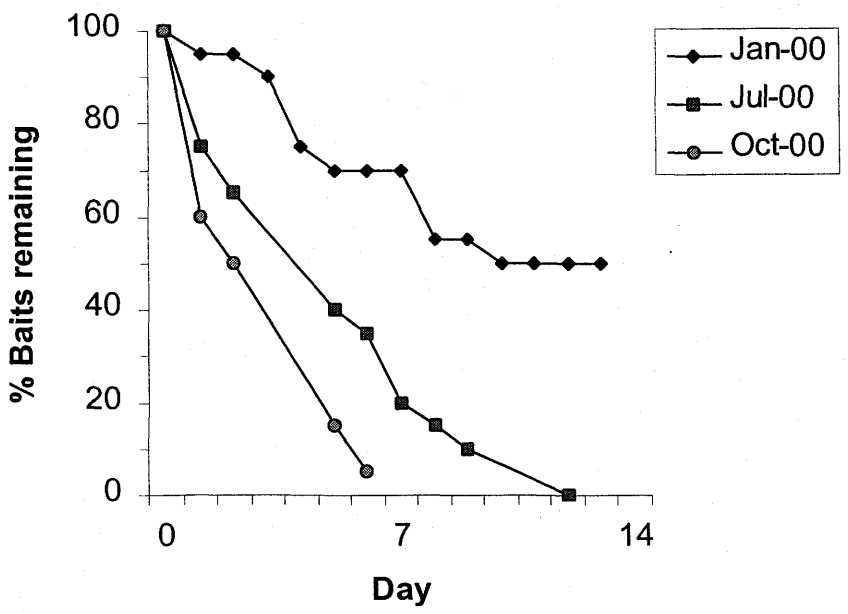

Figure 2. Disappearance of Ramik® Green bait (expressed as % baits remaining) in mesic forest, Hawaii Volcanoes National Park, after hand-broadcast bait applications in January, July, and October 2000.

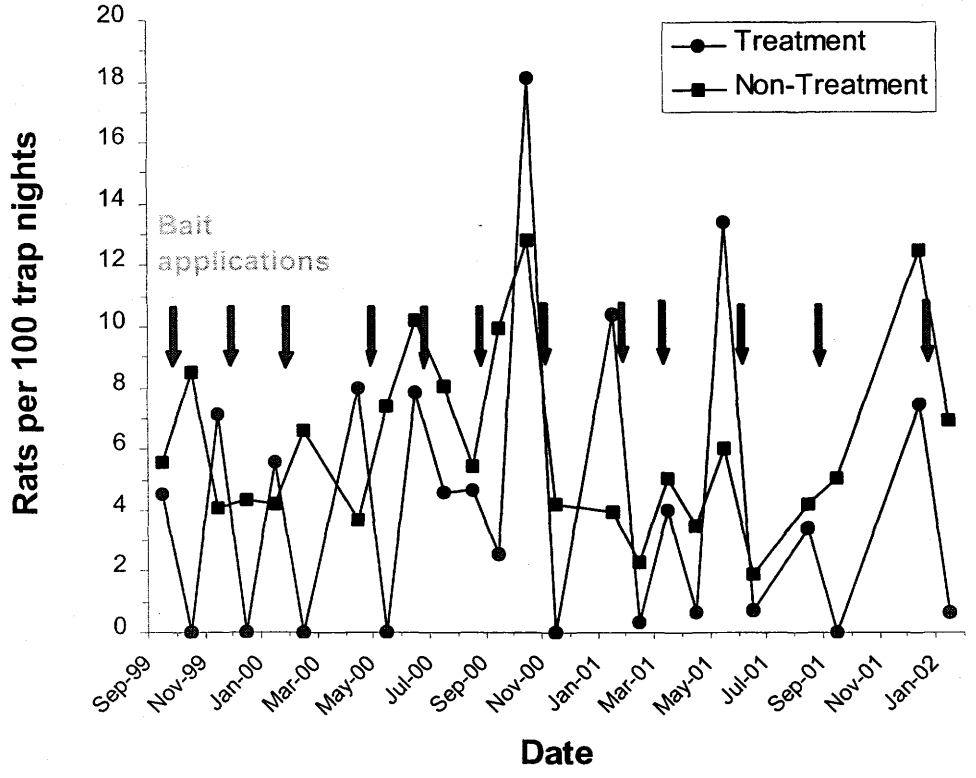

Figure 3. Live captures of rats (per 100 corrected trap-nights) in wet forest, Hawaii Volcanoes National Park (arrows indicate date of bait applications).

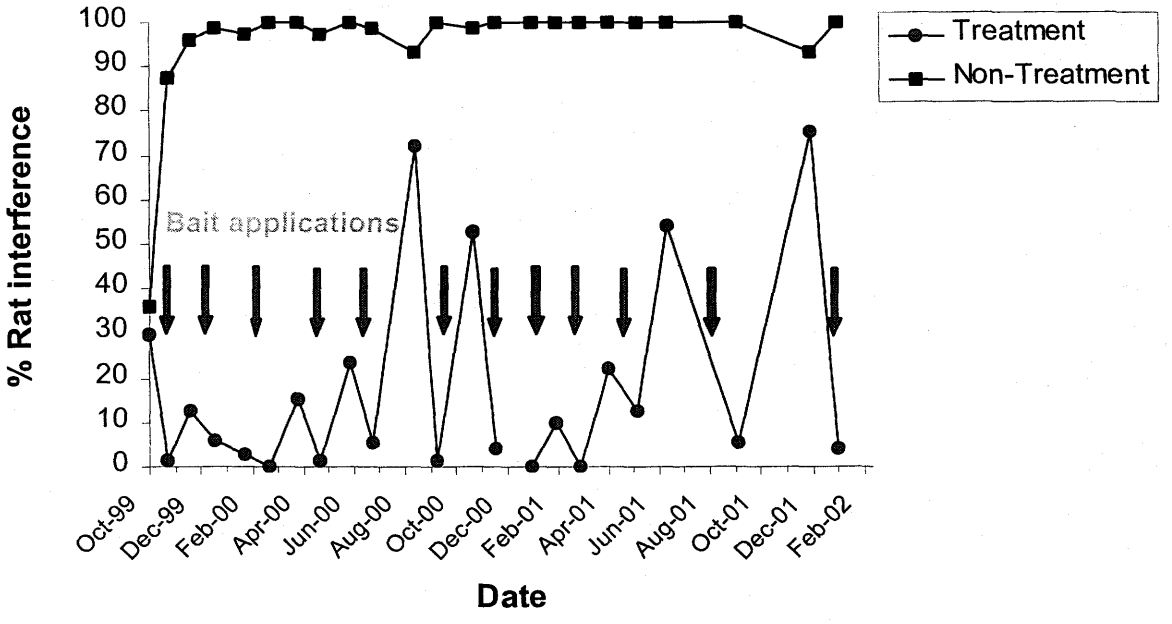

Figure 4. Percentage of non-toxic census bait blocks interfered with by rats in wet forest (arrows indicate date of bait applications).

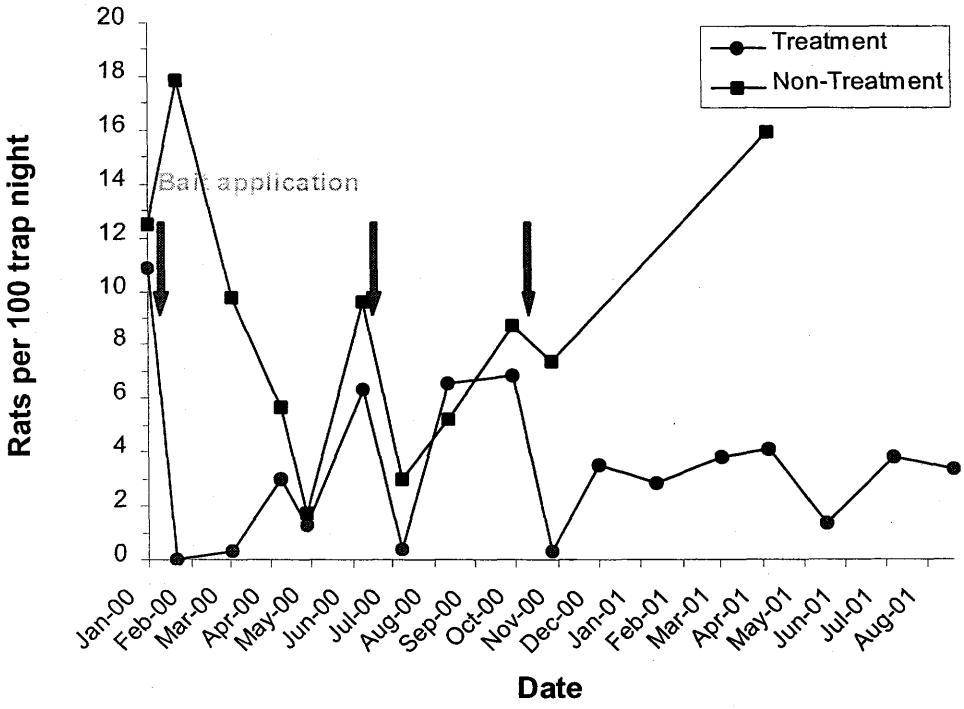

Figure 5. Live captures of rats (per 100 corrected trap-nights) in mesic forest (arrows indicate date of bait applications).

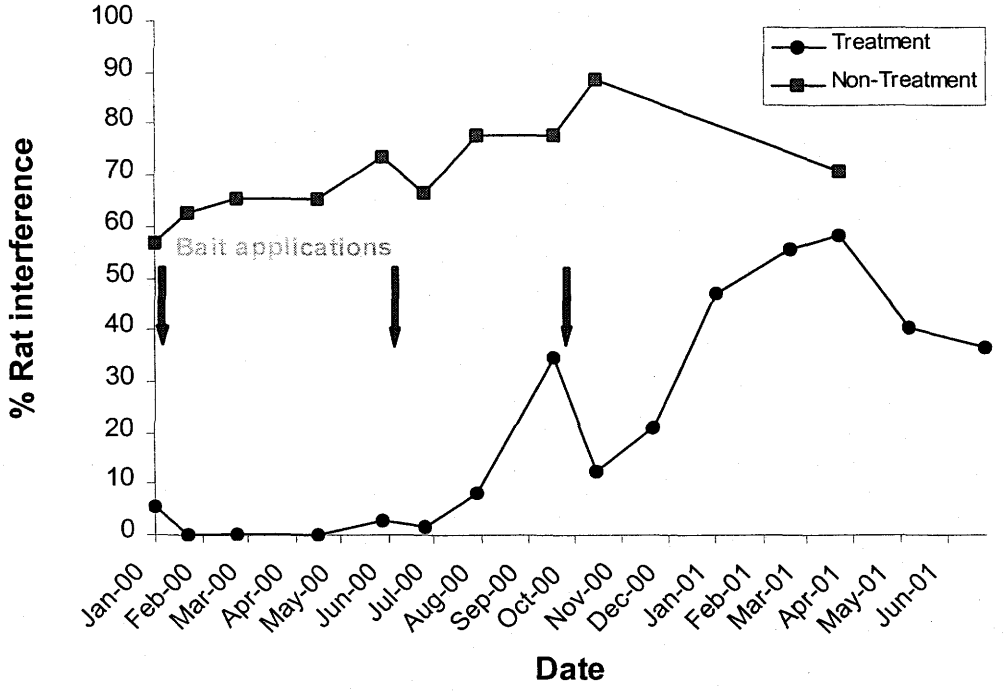

Figure 6. Percentage of non-toxic census bait blocks interfered with by rats in mesic forest (arrows indicate date of bait applications).

X. APPENDICES

Appendix 1. Study protocol QA-02.
Appendix 2. Amendments to study protocol QA-02.
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Appendix 4. Experimental Use Permits (EUP-99-01 and EUP-99-02).
Appendix 5. Concurrence letter from U.S. National Park Service.
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Appendix 7. Concurrence letter from State of Hawaii Division of Forestry and Wildlife.
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Appendix 9. Biological opinion from U.S. Fish and Wildlife Service.
Appendix 10. Environmental assessment report.
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Appendix 12. Ramik® Green label.
Appendix 13. Material safety data sheet.
Appendix 14. End use product tracking form for Ramik® Green bait.
Appendix 15. HACCO certificates of analysis of Ramik® Green bait.
Appendix 16. Malkov and Mach (2002), Genesis Laboratories report.
Appendix 17. Standard operating procedures.
Appendix 18. Dates of bait application and monitoring activities.
Appendix 19. Landcare Research toxicology laboratory analysis reports.
Appendix 20. Dates and results of non-target carcass searches.
Appendix 21. Signs of diphacinone poisoning in radio-collared rats found dead.
Appendix 22. Descriptions of rat nests.
Appendix 23. Ear-tagged rats caught in the treatment plots after bait application.
Appendix 24. Locations where radio-collared rats were found dead
Appendix 25. Diphacinone residues in birds.

Appendix 1. Study protocol QA-02

PACIFIC ISLAND ECOSYSTEMS RESEARCH CENTER
BIOLOGICAL RESOURCES DIVISION
U.S. GEOLOGICAL SURVEY
U.S. DEPARTMENT OF THE INTERIOR

Study Protocol (Aug. 20, 1999)

I. Title:

Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests.

II. Study Directors:

Gerald D. Lindsey, Wildlife Biologist.
David Foote, Ecologist

III. Sponsors:

U.S. Dept. of the Interior, U.S. Geological Survey, Biological Resources Division, Pacific Island Ecosystems Research Center
U.S. Dept. of the Interior, National Park Service, Hawaii Volcanoes National Park
HACCO, Inc., Madison, WI

IV. Background and Justification:

Alien small mammal and invertebrate predators have had devastating impacts on insular environments worldwide, including the Hawaiian Islands. Evolution of the Hawaiian fauna and flora occurred in a relatively high degree of isolation and its members are unusually susceptible to selections pressures from non-native animal species. Native Hawaiian wet forests harbor much of the remaining endemic biological diversity. Mesic forests cover less area than do wet forests and have been much disturbed by human activities, but those in protected areas support a diversity of native woody plant species. However, the role of introduced predators on forest health and ecosystem properties in these habitats is poorly understood. In Hawaii, four species of introduced rodents, the black (Rattus rattus), Polynesian rat (R. exulans), Norway rat (R. norvegicus) and house mouse (Mus musculus) are found in a variety of habitats from sea level to 3050 m elevations (Stone 1985, Tomich 1986). These rodents, together with the introduced feral cat (Felis catus) and Indian mongoose (Hespestes auropunctatus) inhabit forest habitat in varying degrees of sympathy with native Hawaiian forest birds, plants, and invertebrates.

All three rat species, house mouse, feral cat, and mongoose are found in native forests on Hawaii (Sugihara 1997, Tomich 1986, USGS/BRD, unpubl. data). Depredation of eggs, nestlings and adult birds by introduced mammalian predators has been widely postulated as a leading cause of the accelerated decline and extirpation of endemic Hawaiian avian species and

as a major factor limiting present populations of endangered forest birds (Atkinson 1977, Berger 1981, Scott et al. 1986). In addition to eating eggs, nestlings and adult birds, rats prey on native Hawaiian tree snails (Miller and Hatfield 1993) and insect larvae (Sugihara 1997). Rats may also compete for food with the Hawaiian Crow (Corvus hawaiiensis) and Omao (Myadestes obscurus) (Scott et al. 1986) and some endemic insectivorous bird species.

The size, arboreal behavior, and nocturnal habits of black rats cause them to be the greatest rodent threat to native forest birds. Both black and Polynesian rats are known predators to ground and burrow nesting birds (Johnson 1945, Baldwin 1945, Berger 1981, Kepler 1967, Tomich 1986, Woodward 1972). Norway rats are generally restricted to cropland and areas inhabited by humans and are uncommon in forest habitats (Tomich 1986, USGS/BRD unpubl. data).

Seeds and fruits of many endemic plant species are susceptible to predation by rats. The fruits of both rare and common species of Clermontia are eaten by rats in wet forests. In a study carried out in wet montane forests of Maui, Sugihara (1997) reported a high frequency of fruits and seeds of native plants in rat stomachs; plant species identified included Rubus hawaiiensis, Coprosma spp., and Pittosporum spp. Early reports of rat damage in native wet forests included observations of predation on fruits and seeds of the indigenous liana `ie`ie (Freycinetia arborea) (Perkins 1903) and endemic loulu palms (Pritchardia) (Beccari and Rock 1921). The impact of rats on endangered plants in wet forests is not well studied. In mesic forests of Hawaii Volcanoes National Park, black rats damage flowers, fruit, seeds, and bark of the endangered hau kuahiwi tree (Hibiscadelphus giffardianus) (Baker and Allen 1978). Bark stripping and seed predation have also been noted on other mesic forest tree species, including olopua (Nestegis sandwicensis), pilo (Coprosma rhynchocarpa), koa (Acacia koa), ho`awa (Pittosporum hosmeri), sandalwood (Santalum paniculatum), and a`e (Zanthoxylum dipetalum) (Russell 1980, Scowcroft and Sakai 1984, Cuddihy and Stone 1990).

Ebenhard (1988) concluded that the feral cat was the most dangerous predator ever introduced to islands by man, and cited 38 known or probable cases where cats have seriously affected the abundance of prey populations. The role of feral cats as predators on Hawaiian forest birds is poorly known, but several findings suggest that they may be important predators of native Hawaiian birds. Snetsinger et al. (1994) found remains of five banded birds in 30 cat scats collected near Puu Laau on Mauna Kea, even though only a small percentage of birds in the area were banded (USGS/BRD, unpubl. data). Van Riper (1980) watched a feral cat attack and eat a brooding female Palila, and found partial remains of Common Amakihi (Hemignathus virens) and Elepaio (Chasiempis sandwichensis) in the stomachs of a mongoose and two cats collected at Puu Laau (van Riper 1978). Richardson and Woodside (1954) thought that feral cats were serious predators on nesting dark-rumped petrels on Mauna Kea on the island of Hawaii.

Mongooses are not normally arboreal and have poor tree climbing abilities. However, they are adaptable predators and are known to take eggs, young, and adults of eight species of Hawaiian birds Federally listed as endangered (Stone et al. 1995). They may also occasionally kill forest birds which feed on or near the ground or fledglings which spend some time on the ground before becoming proficient in flying.

There are only two methods (trapping and toxicants) available for controlling predators affecting native animal and plant populations in forested areas of Hawaii. Trapping can be an effective short-term nonchemical means of controlling predators in small or limited areas.

Diphacinone (0.005%), a first generation anticoagulant, in two flavors (peanut butter and fish) in all weather bait blocks placed in bait stations has 24c State of Hawaii registration for use against rats in off-shore islands, forests, and other non-crop areas. The fish-flavored bait is also registered against mongoose. However, trapping and use of bait stations are labor intensive and impractical for controlling predators over large conservation areas. Studies at Hakalau Forest National Wildlife Refuge have demonstrated that, while the use of peanut butter-flavored bait placed in bait stations was effective in reducing black rat populations, Polynesian rats appeared reluctant to accept the bait in its present formulation or distribution method (USGS/BRD, unpubl. data). No toxicants are registered for the control of feral cats in Hawaii.

Rats and mongoose are highly sensitive to anticoagulant toxicants. Multiple feedings by rats on diphacinone baits are required and most mortalities generally occurs within 4 - 9 days (Swift 1998). Based on laboratory bioassays, Swift (1998) recommended minimum exposure periods and bait amounts of 7 days and 37.5 g (0.005% diphacinone bait; Ramik[7] Green) for black rats and 6 days and 30.0 g for Polynesian rats for effective control of wild rats in Hawaiian ecosystems. A sublethal dose does not result in poisoning symptoms eliminating the development of bait shyness. The LD_{50} (amount of chemical required to kill 50% of the population) is 0.3-7 mg/kg for rats, 3.0-7.5 mg/kg in dogs, 14.7 mg/kg in cats, 150 mg/kg in pigs, 50-300 mg/kg in mice, 35 mg/kg in rabbits (Extoxnet 1993, IPCS 1995), and 0.18 mg/kg for mongooses (Keith et al. 1990). Birds seem relatively tolerant to diphacinone, the LD_{50} for mallard duck was 3,158 mg/kg (IPCS 1995). The LC_{50} (lethal concentration required to kill 50% of the population) for bobwhite quail was 4,485 mg/kg and for mallard ducks was >10,000 mg/kg in 8-day dietary studies (IPCS 1995).

Keith et al. (1990) fed pigs diphacinone (0.6 mg and 1.5 mg) for short periods (2 to 5 days). All pigs survived and, when sacrificed, symptoms of toxicosis were not evident at necropsy. No diphacinone residues were found in muscle tissues but low levels (0.54 - 0.83 ppm) were found in liver samples. They note that this is well below the therapeutic (5 mg) dose for humans. Even at the highest level, more than ten tons of liver would need to be consumed to attain the human therapeutic dosage. During baiting for mongoose control, a pig killed at the study site by researchers showed no residual diphacinone after laboratory analysis (Keith et al. 1990).

In secondary toxicity studies, Mendenhall and Pank (1980) observed no signs of intoxication in two barn owls (*Tyto alba*) consuming diphacinone-killed rats over a 10 day period. In another test, 3 great-horned owls (*Bubo virginianus*) and 1 saw-whet owl (*Aegolius acadicus*), were fed 2 diphacinone-killed mice daily for 5 days. Three owls died within 14 days, suggesting that a potential hazard could exist under some field conditions (Mendenhall and Pank 1980). Savarie et al. (1979) fed sheep meat containing 2.7 ppm diphacinone to golden eagles in laboratory tests for 5-7 days. All eagles survived but all showed varying degrees of toxicity before recovery. These investigators suggested that a secondary hazard exists for animals that feed repeatedly on contaminated tissue. Under field conditions, however, alternate foods are available reducing the possibility of consumption of contaminated prey.

Lindsey and Mosher (1994) assessed the secondary hazard potential of diphacinone to raptors, particularly the endangered Hawaiian hawk or 'Io and the Hawaiian owl or Pueo, within forested areas. Results from their study suggest that hazards to avian predators from baiting with 0.005% diphacinone bait will be minimal. Dead rats (kill trapped) were located rapidly (= 2.9

days) by mammalian scavengers and raptors did not appear to recognize dead rodents laying on the forest floor as food items. Other rats moving above ground during the day, before and after consuming diphacinone bait, remain under cover, minimizing their exposure to avian predators. Because of the short duration that diphacinone-contaminated rats would be available for scavenging and the availability of a wide range of prey, the potential risk for injury or death for 'Io or pueo, as result of poisoning from diphacinone, would be minimal.

The non-target secondary poisoning hazard resulting from the use of anticoagulants is also reduced by the delay in death of the target species, which allow time for the cleaning of the gut content, metabolism and excretion of the toxicant (Godfrey 1985). Cox and Smith (1992) showed that intake of food and water declined rapidly in anticoagulant-treated, caged *Rattus norvegicus*. Hooker and Innes (1992) reported that black rats poisoned with the anticoagulant brodifacoum maintained normal nocturnal movements and showed no nest change between dawn and dark, suggesting no daytime movements. Most rats died in their nests or under cover, suggesting that few rats dying of anticoagulant poisoning would be found in the open (Hooker and Innes 1992, Lindsey and Mosher 1994).

Over the past two decades, Hawaii Volcanoes National Park (HAVO) has successfully eliminated or reduced feral ungulates and many alien plant species in Special Ecological Areas (SEA). The results of resource monitoring, including population studies of forest birds and rare plants and invertebrates, indicate that control of small mammals is the next major challenge for HAVO resource managers. Introduced rats, mongoose and feral cats have contributed to the loss or decline of many native species in the park, including twelve listed threatened and endangered (T&E) vertebrates (four of which are currently missing from the park). At least half of the nineteen naturally occurring T & E plant species of the Park, as well as most of the native plants listed as "species of concern", have fleshy fruits or large seeds vulnerable to rats. Rat predation on seeds, fruit, and invertebrates contribute to reduced food resources for forest birds, to decreased populations of insect pollinators, and to limited reproduction and spread of some endemic plants. As a consequence, testing multispecies toxicants is ranked as the highest priority for research in the FY1997 Resources Management Plan for the park.

We proposed to evaluate efficacy and safety of control techniques and bait delivery systems in wet and mesic forest habitats within SEA's at HAVO to generate supporting data needed for state and federal regulatory approval for aerial broadcast bait application.

V. Objectives:

1. Determine efficacy of hand broadcast baiting with 0.005% diphacinone bait for the control of rats in wet forest habitat.

2 . Determine efficacy of hand broadcast baiting with 0.005% diphacinone bait for the control of rats in mesic forest habitat.

3. Determine efficacy of aerially broadcast baiting with 0.005% diphacinone bait.

4. Determine disappearance rate from the forest floor of diphacinone pellets.

5. Monitor secondary hazard potential of hand and aerially broadcast 0.005% diphacinone bait.

6. Monitor the impact of rodent predation on and recovery of representative populations of native flora and fauna following control applications

VI. Study Locations:

The 10-acre hand broadcast application trial will be conducted within the Hawaii Volcanoes National Park. The wet forest site will be in the southwest corner of `Ola`a Forest within the Koa Management Unit at approximately 1,200 m elevation. This 800 ha unit was fenced in 1990 and has been free of feral pigs since 1994. The forest in this section of `Ola`a is composed of an open canopy or scattered large `ohi`a trees (Metrosideros polymorpha) with an open understory of mixed native trees and a dense lower layer of tree ferns (Cibotium spp.) (Jacobi *et al.* unpublished). The tree fern layer is 2-5 m tall and has a dense cover of 80-90%. Ground cover consists primarily of native ferns, shrubs, and sedges, but a few alien plants are also common here, particularly yellow Himalayan raspberry (Rubus ellipticus) and banana poka Passiflora mollissima).

The mesic forest component of this trial will be conducted in Kipukas Puaulu and Ki, ancient kipukas of deep ash soil surrounded by lava of the late prehistoric Keamoku flows. Both kipukas are on the lower slope of Mauna Loa at 1,200-1,360 m elevation, and are approximately 8 km west of the wet forest study site of `Ola`a. Vegetation of the central part of the kipukas is composed of a tall koa/`ohi`a/soapberry (Acacia koa/ Metrosideros polymorpha/Sapindus saponaria) forest. Ground cover is dominated by native ferns and herbs where the forest canopy is dense, but blackberry (Rubus argutus) and alien grasses, such as meadow ricegrass (Ehrharta stipoides) and Paspalum spp., are common in some areas. Kipuka Ki also contains some Jerusalem cherry *(Solanum pseudocapsicum)*. Large patches of open grassland with scattered trees also occur in the kipukas (Mueller-Dombois and Lamoureux 1967). Kipuka Ki was fenced against cattle in 1940s, and has been free of feral pigs since the mid-1980s. Kipuka Puaulu was fenced against cattle in 1930s and has been free of feral pigs since the mid-1960s.

The aerial broadcast application trial will be conducted within the Hawaii Volcanoes National Park. Specific study sites will be selected following completion of the hand broadcast application trials.

VII. Methods:

A. Procedure

a. TEN-ACRE HAND BROADCAST BAIT APPLICATION

1. Study area. Two 4-ha (200 x 200 m) study plots will be established in each the wet and mesic forest sites in Hawaii Volcanoes National Park. A 25-m x 25-m grid will be established over each study area. In Olaa forest, the control plot will be separated from the treated plot by at least 400 m. Alien plant control is underway in the part of `Ola`a

Forest adjacent to Wright Road, so the wet forest study plots will be placed at least 500 m in the interior of the forest. Existing transects Nos. 16 and 18 in `Ola`a Forest will be utilized to access the study plots, which will be selected along two transects in an area of relatively homogeneous vegetation. The treatment plot will be along transect 18 and the control plot along transect 16. In the mesic forest, the treatment plot will be established in Kipuka Ki and the control plot in Kipuka Puaulu, 1.6 km east of Kipuka Ki. Warning signs will be erected when diphacinone bait is applied.

2. Livetrapping. Eighty-one basket live traps will be placed at 25-m intervals on grid lines spaced 25 m apart within each 4-ha study plot to obtain indices of rat densities pre- and post-treatment (SOP BRD-04). All traps (in a closed condition) will be placed at trap locations two weeks before actual trapping to allow rats time to become accustomed to the traps. Two weeks before toxic pellets are applied to the treatment areas, trap locations in each study plot will be prebaited with shredded coconut three days prior to trap activation, then traps will be baited with coconut chunks and opened for four consecutive nights. All rats captured will be identified to species, sexed, weighed, eartagged and released (SOP BRD-09) . Traps will also be opened 1 week, 2 months, and 4 months after the first and each subsequent hand broadcast baiting series to determine efficacy and rat re-invasion rates. For each forest type (mesic and wet), rat capture rates pre- and post-treatment in the control and treatment plots will be calculated following the method by Nelson and Clark (1973) and compared by Chi-square analysis.

3. Census blocks. One week before the treatment plots are baited, 72 CENSUS™ non-toxic bait blocks will be placed on a 25- x 25-m spacing within each 4-ha treatment and control plot(SOP BRD-11). Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5 m, 62.5 m, etc.) for 2 nights before baiting, and at 2 weeks, 2 months, and 4 months following each toxic baiting series to determine efficacy and rat re-invasion rates. Blocks will be examined daily for 2 days for rodent gnawing, and slug and snail feeding. For each forest type (mesic and wet), the proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared using Chi-square analysis.

4. Radio telemetry. Before the initial bait application, 10 to 15 rats within each treatment plot and 10 to 15 rats within the each control plot will be fitted with transmitters (SOP BRD- 10). Each rat will be monitored daily for at least three consecutive days immediately before baiting to ensure the rat is alive and active (SOP BRD-13). Each rat will be monitored daily for two weeks following bait application to determine its fate. Nighttime monitoring will determine movement activity of radio-marked rats. Daytime nest sites of each rat will be determined at least twice a week. Nest locations will be recorded, nest material identified, and the nest will be measured (width, depth, inside diameter). Nests will be collected by placement in a plastic ZipLok bag. Woody trees and tree ferns containing rat nests will be identified, measured (trunk diameter at 4.5'), and the height of the nest in the tree determined.

We will attempt to locate and remove all radio-collared rats dying during the study. Dead rats will be examined for green bait within their stomach and intestines, and for hemorrhaging characteristic of anticoagulant poisoning. All rat carcasses will be placed in marked containers, frozen, and retained for possible diphacinone analysis. One month following bait application, radio-collared rats still alive will be live trapped and their radios removed. For each forest types (mesic and wet), the proportion of radio-collared rats surviving in post-treatment will be compared by Chi-square analysis.

5. Kill trapping - House mouse (*Mus musculus*). In each 4-ha study plot, 40 mouse traps (2 traps at a location) will be placed at 10 m intervals along one transect within each 4-ha study plot to estimate mouse densities pre- and post-treatment. Traps will be opened 3 weeks before toxic bait is applied, 2 weeks after toxic bait is applied, then at 2-month interval after each hand broadcast toxic baiting series. During each trapping session, traps will be baited with coconut chunks, opened, and examined daily for captures for 2 days. Traps will be rotated to a new transect before each trapping session. Mouse capture rates pre- and post-treatment in the control and treatment plots will be calculated following the method by Nelson and Clark (1973) and compared by Chi-square analysis.

6. Diphacinone Baiting. One fish-flavored formulations (Ramik®, HACCO, Inc., Madison, WI) of 0.005% diphacinone pellet bait (mean pellet size = 5.8 g) will be tested. Prior to the actual 0.005% diphacinone bait application, all personnel will practice hand-dispensing untreated pellets (rat food pellets or untreated bait pellet) of similar size to simulate the selected application rate along 10 m transects in an open field.

One 4-ha treatment plot within the wet forest (Olaa forest - transect 16) and one 4-ha treatment plot within the mesic forest (Kipuka Ki) will be hand broadcast baited with the Ramik® bait. Each treatment plot will be flagged at 10 m intervals (first transect placed at 5 m from the edge of the plot) to facilitate personnel access for bait application. The 0.005% diphacinone bait pellets will be applied when the ground is reasonably dry and weather predictions call for less than 2 -inch rain in the next five days. Bait application rate will be between 11.22 kg/ha (10 lbs/acre) and 33.64 kg/ha (30 lbs/acre) and will follow recommendation from controlled field trials (to be conducted May - July 1999) using a nontoxic bait containing a biological marker to determine the optimum bait application rate. The other 4-ha plot within each forest will not be baited to serve as a control.

For each series of bait application, one-half of the bait will be hand broadcast on Day 1 and one-half of the bait will be hand broadcast between Day 3 and Day 7 (e.g., for an application rate of 33.62 kg/ha (30 lbs/acre), one-half (16.81 kg/ha or 15 lbs/acre) will be hand broadcast on Day 1 and 16.81 kg/ha (15 lbs/acre) will be hand broadcast between Day 3 and Day 7). Timing of the second application rate will depend upon the disappearance rate of marked pellets (see below). During the following one year period, hand broadcast toxic bait applications will be repeated in the treatment plots every 2-3

months or when rat populations recover to pretreatment levels, which ever is longer. A maximum of 5 series of toxic bait applications will be applied to each treatment plot.

7. Bait Disappearance rate. Immediately after each toxic bait application series, the location of 10 individual baits will be marked with numbered, colored wire flags at four separate sites within each 4-ha baited area. Location of pellets will equal the density of pellets per area from actual hand broadcast distribution. Each numbered, colored wire flag will be placed at a compass bearing of 360 degrees and touching the pellet. Each pellet will be examined daily for 14 days or until eaten or the bait disintegrates. Data will be recorded on disappearance, consumption by rats, and feeding by slugs, snails and other invertebrates.

8. Environmental conditions. Rain gauges and minimum/maximum thermometers will be established in the treatment plot of each study area. Daily rainfall and temperature will be recorded by 10 a.m. each morning for 2 weeks after each bait distribution series. Rainfall will be recorded at weekly intervals between bait applications.

9. Non-target hazards - Vertebrates. Avian predators seen within the study areas will be recorded throughout the study. Location of radio marked rats dying during the study will be recorded to determine if the carcasses are accessible to avian predators. Four transects (400 m long x 5 m wide and spaced 25 m apart; 10% of each total study plot) within each treatment and control plot will be walked to search for non-target mortality 2 weeks before, and 2 weeks and one month after each bait application series during the one year period. Each transect will extend 100 m beyond each end of the study plots. Any carcass discovered will be recorded to species, weighed, sexed, placed in individually marked containers, frozen and retained for possible residue analysis.

We anticipate that our study area will be within the home range of several radio-marked `Io (*Buteo solitarius*) involved in a separate study. Radios on these `Io carry a mortality signal. If `Io with radios are within or adjacent our study areas, we will monitor these `Io before, during and after the toxicant drop to determine use of the study area by these birds.

We will collect tissue and/or whole body samples from four introduced bird species: Japanese white-eye (*Zosterops japonicus*), red-billed leiothrix (*Leiothrix lutea*), northern cardinal (*Cardinalis cardinalis*) and kalij pheasant (*Lophura leucomelana*) for diphacinone residue analysis. Birds will be collected using mist nets or shooting (kalij pheasant) in each treatment plot 1 - 4 weeks after baiting. Samples will be placed in marked plastic bags, frozen, and retained for possible residue analysis

10. Monitoring native flora and fauna recovery. Twenty seed traps (to exclude predators) will be placed in each control and treatment area to determine seed fall and seed predation under target tree species thought to be vulnerable to rat predation. Tree species selected for this study in the wet forest will include Coprosma spp. Myrsine

lessertiana, Melicope spp., and possibly Cheirodendron trigynum. In the mesic forest site, target native tree species will be selected from those known to be predated by rats: Hibiscadelphus giffardianus, Pittosporum hosmeri, Pisonia brunoniana, Coprosma rhynchocarpa, and Pipturus albidus. Seed traps will be made from pvc pipe constructed as a frame standing on four legs raised above the ground. A fine nylon mesh bag will be attached to the frame to catch seeds. The size of the traps and plots will be at least 50 by 50 cm. Twenty open seed plots of the same size will be established at ground level to monitor seed loss of the same tree species. One seed plot will be placed within 1 m of each seed trap. Seed traps will be examined for seeds monthly. In the rat-proof seed traps, seeds will be examined for rat damage done in the trees prior to seed fall. Seed loss will be compared by Chi Square analysis.

Seeds of select native tree species will be germinated in a greenhouse and outplanted inside and outside enclosures to determine predation over a one-year period. One hundred forty seedlings of each species selected (100 in the treatment area and 100 in the control area) will be planted in rat-proof enclosures(5 in each of 20 replicates), and the same number will be planted outside the enclosures in both treatment and control areas. Seedlings outside the rat-proof enclosures will be protected from Kalij pheasants (Lophura leucomelana) by a barrier passable to rats. Another 100 seedlings will be planted inside slug-proof barriers ate each study site. Fate of seedlings will be monitored weekly. Predation on seedlings will be compared using Chi Square analysis. Species to be outplanted as seedlings in the wet forest will include Clermontia parviflora, Cheirodendron trigynum and Coprosma spp. Species outplanted in the mesic forest will be Clermontia hawaiiensis, Hibiscadelphus giffardianus, Pittosporum hosmeri, Pisonia brunoniana, Cheirodendron trigynum, and possibly Pipturus albidus. Existing seedlings of some hard-to-propagate native species, such as the loulu palm or Pritchardia beccariana, may be monitored in the same way as outplanted seedlings, if they occur within the study plots.

Up to 50 each of flowers, fruit, and buds on selected native tree species will be marked in each of the control and treatment areas. These will be monitored weekly to determine their fate. Seeds of selected species known to be attractive to rats, such as Pittosporum hosmeri, Hibiscadelphus giffardianus, and Pisonia brunoniana, will be glued or tethered to trees in the treatment and control plots of the mesic forest and will be examined at intervals of two days to detect rat predation.

Ten vegetation plots (10 x 20m) will be randomly selected within each study site to evaluate stand structure of native forest trees before and after treatment to remove rats. A similar set of plots will be established in untreated study sites to act as controls.

Soil and litter macroinvertebrates will be compared between treatment and control areas using randomly placed pitfall traps (16 oz. Reynolds plastic cups with rain shelter). Trap contents will be collected weekly and sorted. The taxonomic composition and biomass of arthropods and other invertebrates will be summarized for each trap.

b. AERIAL BROADCAST BAIT APPLICATION

1. Study area. Two 20.2 ha (50 acres; 450 x 450 m) study plots will be established in either the wet or mesic forest within HAVO. Selection of forest type will be established based on the results of the hand broadcast bait application study. Forest habitat will be similar to that used for the hand broadcast bait application described above.

2. Livetrapping. In each 20 ha study plot, 144 basket live traps will be placed at 25 m intervals on transects spaced 25 m apart to obtain indices of rat densities pre- and post-treatment (SOP BRD-04). All traps (in a closed condition) will be placed at trap locations two weeks before actual trapping to allow rats time to become accustom to the traps. Two weeks before the treated area is baited, trap locations in each study plot will be prebaited with shredded coconut three days prior to trap activation, then traps will be baited with coconut chunks and opened for four consecutive nights. All rats captured will be identified to species, sexed, weighed, eartagged and released (SOP BRD-09). Traps will also be opened 2 week, 3 months, and 6 months after aerial bait application to determine efficacy and rat re-invasion rates. Rat capture rates pre- and post-treatment in the control and treatment plots will be calculated following the method by Nelson and Clark (1973) and compared by Chi-square analysis.

3. Census blocks. One week before each treatment plot is baited, 132 CENSUS^{TM} non-toxic bait blocks will be placed on a 25- x25-m spacing within each 20 -ha plot (SOP BRD-11). Blocks will be placed on transects in between live trap locations (i.e., 25 m, 75 m, 125 m, etc.) for 2 nights before baiting, and at 3 weeks, 3 months and 6 months following baiting to determine efficacy and rat re-invasion rates. Blocks will be examined daily for 2 days for rodent gnawing, and slug and snail feeding. The proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared using Chi-square analysis.

4. Radio Telemetry. Before the initial bait application, up to 25 rats within each treatment and up to 25 rats within each control plot will be fitted with transmitters (SOP BRD- 10). Each rat will be monitored daily for at least 3 consecutive nights immediately before baiting to ensure the rats are active and alive (SOP BRD-13). Each rat will be monitored daily for two weeks following bait application to determine its fate. Nighttime monitoring will determine movement activity of radio-marked rats. Daytime nest sites of each rat will be determined at least twice a week. Rat nest locations will be recorded, nest material identified, the nest will be measured (width, depth, inside diameter). Nests will be collected by placement in a plastic ZipLok bag. Woody trees and tree ferns containing rat nests will be identified, measured (trunk diameter at 4.5'), and the height of the nest in the tree determined.

We will attempt to locate and remove all radio-collared rats dying during the study. Dead rats will be examined for green bait within their stomach and intestines, and for

hemorrhaging characteristic of anticoagulant poisoning. All rat carcasses will be placed in marked containers, frozen, and retained for possible diphacinone analysis. One month following bait application, radio-collared rats still alive will be live trapped and their radios removed. The proportion of radio-collared rats surviving in post-treatment will be compared by Chi-square analysis.

5. Kill trapping - House mouse (Mus musculus). In each 20-ha study plot, 54 mouse traps (2 traps at a location) will be placed at 10 m intervals along one transect to estimate mouse densities pre- and post-treatment. Traps will be opened 2 weeks before toxic bait is applied, 4 weeks after toxic bait is applied, then at 3 & 6 month interval after each hand broadcast toxic baiting series. Traps will be rotated to a new transect before each trapping session. During each trapping session, traps will be baited with coconut chunks, opened, and examined daily for captures for 2 days. Mouse capture rates pre- and post-treatment in the control and treatment plots will be calculated following the method by Nelson and Clark (1973) and compared by Chi-square analysis.

6. Diphacinone Baiting. One fish-flavored formulation (Ramik® Green, HACCO, Inc., Madison, WI) of 0.005% diphacinone pellet baits (8 g/pellet) will be tested. The 20 ha treatment plot will be aerially broadcast baited with the fish-flavored diphacinone bait. The other 20 ha plots will not be baited to serve as a control. We anticipate that ½ of the bait will be aerially broadcast on Day 1 and ½ of the bait will be aerially broadcast between Day 3 and Day 7. Bait application rate (kg per ha) and duration between bait applications will follow recommendation from controlled field trials using a nontoxic bait containing a biological marker to determine the optimum bait application rate and the results of the hand broadcast baiting trials discussed above. Bait pellets will be aerially applied when the ground is reasonably dry and weather predictions call for less than 2-inch rain in the next 5 days.

7. Bait Disappearance rate. Immediately after each bait application, the location of 20 individual baits will be marked with numbered, colored wire flags at four separate sites within each treatment plot. Location of pellets will equal the density of pellets per area from actual aerially broadcast distribution. Each numbered, colored wire flag will be placed at a compass bearing of 360 degrees and touching the pellet. Each pellet will be examined daily or 14 days or until taken or the bait disintegrates. Data will be recorded on disappearance, feeding by slugs, snails and other invertebrates, and consumption by rats.

8. Environmental conditions. Rain gauges and minimum/maximum thermometers will be established at the study area. Daily rainfall and temperature will be recorded by 10 a.m. each morning for 2 weeks after each bait distribution series. Rainfall will be recorded at weekly internals between bait application series.

9. Non-target hazards. Avian predators seen within the study areas will be recorded throughout the study. Location of radio marked rats dying during the study will be

recorded to determine if the carcasses are accessible to avian predators. Four transects (650 m long x 5 m wide and spaced 50 m apart; 6.4% of each study plot) within the treatment and control plot will be walked to search for non-target mortality 2 weeks before, and 2 weeks and one month after bait distribution. Each transect will extend 100 m beyond each end of the study plots. Any carcass discovered will be recorded to species, weighed, sexed, placed in individually marked containers, frozen and retained for possible residue analysis.

We anticipate that our study area will be within the home range of several radio-marked `Io (*Buteo solitarius*) involved in a separate study. Radios on these `Io carry a mortality signal. We will monitor these `Io before, during and after the toxicant drop to determine use of the study area by these birds.

We will also collect tissue and/or whole body samples from four introduced bird species: Japanese white-eye (*Zosterops japonicus*), red-billed leiothrix (*Leiothrix lutea*), northern cardinal (*Cardinalis cardinalis*) and kalij pheasant (*Lophura leucomelana*)-- for diphacinone residue analysis. Birds will be collected by shooting and/or mist netting 1 - 4 weeks after baiting.

B. Identification of test, control, and reference substances, mixtures of substances with carriers, mateials, and/or devices to be used or tested.

1. Ramik® Green fish flavored weather-resistant rodenticide
   Compressed cereal grain: >98.995%
   Sodium Saccharin (CAS No. 128-44-9): <1.0%
   Diphacinone (CAS No. 82-66-6): 0.005%
2. Census™ Bait Block (nontoxic rodent indicator)
3. Wire-cage Haguruma7 live traps (13 x 21 x 27 cm)
4. Victor mouse kill traps (10 x 4.5 cm)
5. Holohil rat radio transmitters (4.2 g)

C. Test substance accountability

Ramik Green rodenticide will be stored in a locked room within a locked building (Bldg 216) at the Hawaii Volcanoes National Park, Volcano, Hawaii.

D. Experimental Design and statistical analyses

See Section VII, A.2, 3, 4, and 10. Catch per unit effort, adjusted for sprung traps, percent census blocks gnawed, percent radio-collared rats alive, seed loss, and predation on seedlings between the control and treatment study sites in each the wet and mesic forests will be compared by Chi-square analysis. Treatment and control sites in the wet and mesic forest were selected by random selection.

E. Environmental conditions

Rain gauges and minimum/maximum thermometers will be established at Kipuka Ki and Olaa forest. Daily rainfall and temperature will be recorded by 10 a.m. each morning for

2 weeks after each toxic bait application series. Rainfall will be recorded at weekly intervals between bait application series.

F. Records

Field notes and data will be recorded with pencil on all-weather writing paper (Rite in the Rain®, J. L. Darling Corp., Tacoma, WA) that shed water and can be used in wet, muddy, and heavily vegetated study locations where use of permanent ink pens and markers is not possible. Strict GLP record keeping guidelines will be adhered to.

1. Diphacinone bait application
    - Date and time of application
    - Study plot number and location
    - Weather conditions during application
    - Name of applicators
    - Name of bait applied
    - Quantity of bait distributed per acre
    - Pounds of bait applied per 4 ha study plot
    - Method of bait application

2. Rat live captures
    - Capture date
    - Capture location (plot, transect, and trap number)
    - Rat species
        - Ear tag numbers
        - Sex and weight
        - Reproductive condition
            - Males - Testes ascended or desended
            - Females - Vagina inperforate or perforate
                - Visibly pregnant
    - Name of recorder

3. Radio Telemetry
    - Date of radio attachment
    - Location (plot, transect, and trap number) of rat
    - Species of rat
    - Ear tag numbers
    - Sex and weight
    - Transmitter number
    - Transmitter frequency
    - Name of person attaching radio
    - Location and activity (moving/not moving) of radio-collared rat
        - Date and time
        - Rat number and transmitter number
        - Plot number

Activity (moving/not moving)
Nest site (Plot No., transect, compass bearing, distance)
Name of recorder

4. Bait disappearance and degradation
   Date and time
   Weather conditions
   Condition of each individual bait
      Bait location (plot and bait number)
      Percent eaten by rodents or invertebrates
      Bait disappearance
      Bait weathering
   Name of recorder

5. Census blocks
   Date and time
   Block location (plot, transect, and block number)
   Percent of block gnawed by rodents and/or invertebrate
   Name of recorder

6. Kill trapping
   Date of trap placement
   Capture date
   Species and sex
   Capture location (plot, transect, and trap number)
   Name of recorder

7. Non-target hazards
   Avian predators
      Date of observation of avian predators
      Location (transect and coordinates within plot)
      Activity of avian predator
      Name of recorder
   Transect searches
      Date and time
      Plot and transect number
      Name of person walking transect
      Location of carcass
      Species, sex, and weight
   Collection of specimens
      Collection date
      Collection method
      Location (transect and coordinates within plot)
      Species

Name of recorder

8. Environmental conditions
   Date and time
   Plot location and number
   Rainfall
   temperature (minimum, maximum, and ambient)

G. Authorities and permits

Written approval obtained from the State of Hawaii, Department of Agriculture, Pesticide Division (EPU-99-01 and EPU-99-02) on May 5, 1999, and from U.S. Dept. of the Interior, National Park Service, Hawaii Volcanoes National Park on [Environmental Assessment dated ____] to conduct this study. U.S. Fish & Wildlife Service's conducted a Section 7 consultation dated Aug. 6, 1999.

H. Standard operating procedures

SOP BRD-04: Live trapping rats (*Rattus* spp.)
SOP BRD-09: Handling, weighing, and ear-tagging live rats (*Rattus* spp.) under field conditions.
SOP BRD-10: Placing radio transmitters on rats (*Rattus* spp.) under field conditions.
SOP BRD-11: Placing non-toxic census blocks in the field to estimate rat (*Rattus* spp.) densities.
SOP BRD-12: Evaluation of rodenticide pellets for degradation and disappearance.
SOP BRD-13: Radio tracking techniques for marked rats (*Rattus* spp.).

I. Analytical chemistry

The Ramik® Green diphacinone will be assayed for diphacinone by the U.S. Department of Agriculture, Animal & Plant Health Inspection Service, National Wildlife Research Center.

J. Bait formulation

This protocol does not involve the formulation of toxic baits.

VIII. Animal Care and Use:

Date of IACUC Approval:

A. Test system.

Black rats, Polynesian rats, and Norway rats will be captured by live traps, ear tagged (some fitted with radio transmitter) and released at the capture site. House mice will be captured by live and snap (kill) traps. Mice live captured will be immediately released

B. Identification of test system.

Black, Polynesian, and Norway rats, and house mice are the only rodent species found in the study area. Rats will individually marked with one numbered ear tag in each ear.

C. Rationale for involving animals, and appropriateness of species, and for numbers.

See Section IV. Rodents (*Rattus* spp.) are the species for which the toxicant and baiting method is being evaluated because of their depredation on Hawaiian native plants and animals. Mice are also present in native ecosystems and may eat broadcast bait. We cannot predict the number, weights, sexes, or ages of animals that we will capture in our live and snap traps or that will consume toxic baits.

D. Source.

Wild rodents in mesic and wet forests within the Hawaii Volcanoes National Park.

E. Trapping

SOP BRD-04

F. Handling/restraint

SOP BRD-09
SOP BRD-10
SOP BRD-11

G. Transport - N/A

H. Housing/Maintenance - N/A

I. Carrier/diet/contaminants - N/A

J. Route of administration.

Orally; baits are intended for ingestion.

K. Dosage

Ramik® Green compressed cereal grain pellets treated with 0.005% concentration of diphacinone will be hand or aerial broadcast in the field at a sowage rate of 11.22 - 33.64 kg/ha (10 - 30 lbs/acre).

L. Quarantine - N/A

M. Disposition of animals

All recovered animals will be retained at USGS/BRD Pacific Island Ecosystems Research Center's Kilauea Field Station or disposed of by burial at the study site.

N. Prior studies

We have searched the scientific literature, as well as communicated with other biologists and bait manufactures in this field, and have discovered no previous studies evaluating broadcast applications of Ramik® Green containing 0.005% diphacinone in native ecosystems similar to Hawaii. The protocol described herein does not duplicate previous experiments.

O. Pain

1. Alternative procedures.
   Live trapping and handling of rats will not result in pain to the animals. House mice will be killed instantly in snap trap. Rodents ingesting diphacinone baits will be allowed to die naturally from the effects of the toxicant. Diphacinone ingested by rats acts by inhibiting enzymes involved in blood clotting and death is caused by hemorrhaging. Evaluation effectiveness of experimental toxicants under field conditions is required before obtaining State and Federal registration of the toxicant and application method.
2. Justification for withholding sedatives or analgesics. Free-ranging rodents consuming diphacinone will die within 3 to 7 days. Mice captured in snap traps generally die instantly. Administering sedatives or analgesics to these individuals is not possible in the field.
3. Consultation with attending Veterinarian.
4. Euthanasia. Animals found alive in snap traps will be immediately euthanized by cervical dislocation. Target alien birds captured in mist nets will be euthanized using CO_2.

IX. Endangered Species Act compliance

Is there a possibility that the study, as proposed, will or may affect threatened or endangered species?

Yes: X No:

A Section 7 consultation on experimental use of diphacinone pellets in Hawaii Volcanoes National Park was conducted by the U.S. Fish & Wildlife Service (Service) Aug. 6, 1999. The only federally listed species in the action area that may be adversely affected by diphacinone is the Io (*Buteo solitarius*). The Service's biological opinion was that hand-broadcast baiting of diphacinone, as proposed, is not likely to jeopardize the continued existence of the Io. The Section 7 consultation did not rule on aerial baiting of diphacinone.

X. National Environmental Policy Act compliance

Does the study, as proposed, have the potential for significant impact on the environment?

Yes:____ No:__X__

XI. Employee safety

USGS/BRD/PIERC safety regulations will be followed. Routine safety procedures will be followed and appropriate protective equipment will be worn by employees handling and distributing bait, and trapping and handling rodents.

XII. Schedule

Proposed Experiment Start Date:	Oct 1999
Proposed Experiment Termination Date:	Oct 2001
Proposed Study Completion Date:	July 2002

XIII. Staffing:

Position (FY 99 - FY02)

USGS/BRD	Wildlife Biologist	0.5
	Ecologist	0.2
	Botanist	0.2
	Biological Technician	0.75
	Biological Technician	0.5
	Intern	0.75
	Intern	0.75
	Total	3.15

XIV. Cooperators and consultants:

Gerald D. Lindsey
USGS/BRD/PIERC
Kilauea Field Station
P.O. Box 44
Hawaii National Park, HI 96718

David Foote
USGS/BRD/PIERC
Kilauea Field Station
P.O. Box 44
Hawaii National Park, HI 96718

Judith Thompson
HACCO, Inc.
Registration Manager, Rodenticides
P.O. Box 7190
Madison, WI 53707

XV. Staff Qualifications

All study participants have documentation on file which verifies their qualifications for the work they will perform in this study.

XVI. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the Pacific Island Ecosystems Research Center at Kilauea Field Station, P.O. Box 44, Hawaii National Park, Hi 96718.

XVII. References

Atkinson, I. A. E. 1977. A reassessment of factors, particularly *Rattus rattus* L., that influenced the decline of endemic forest birds in the Hawaiian islands. *Pac. Sci.* 31:109-133.

Baker, J. K., and M. S. Allen. 1978. Roof rat depredations on Hibiscadelphus (Malvaceae) trees. Pp. 2-5 *in* C. W. Smith (ed.). Proc. 2nd Hawaii Volcanoes National Park Natural Sciences Conf., Honolulu.

Baldwin, P. H. 1945. The Laysan Rail. Audubon 47:383-348.

Beccari, O., and J. F. Rock. 1921. A monographic study of the genus Pritchardia. Memoirs of the B. P. Bishop Museum Vol. 8, No. 1. Bishop Museum Press, Honolulu.

Berger, A. J. 1981. Hawaiian birdlife, 2nd ed. Univ. Hawaii Press, Honolulu.

Cox, P, and R. H. Smith. 1992. Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behaviour. Pp. 165-170 in J. E. Borrecco and R. E. Marsh (eds.), Proceedings of te 15th Vertebrate Pest Conference, Univ. Calif., Davis.

Cuddihy, L. W., and C. P. Stone. 1990. Alteration of native Hawaiian vegetation. Cooperative National Park Resources Studies Unit, University of Hawaii. University of Hawaii Press, Honolulu.

Ebenhard, J. 1988. Introduced birds and mammals and their ecological effects. *Swedish Wildl. Res.* 13:5-107.

Extoxnet. 1993. Diphacinone. Cooperative Extension Office, Cornell Univ.

Godfrey, M. E. R. 1985. Non-target and secondary poisoning hazards of Asecond generation@ anticoagulants. Acta Zool. Fennic 173:209-212.

Hooker, S. and J. Innes. 1995. Ranging behaviour of forest-dwelling ship rats, Rattus rattus, and effects of poisoning with brodifacoum. New Zealand J. Zool. 22:291-304.

IPCS. 1995. Environmental Health Criteria 175: Anticoagulant rodenticides. World Health Organization, Geneva.

Johnson, M. S. 1945. Rodent control on Midway Islands. U. S. Naval Med. Bull. 45:384-398.

Keith J. O., D. N. Hirata, D. L. Espy, S. Greiner, and D. Griffin. 1990. Field evaluation of 0.00025% diphacinone bait for mongoose control in Hawaii. Denver Wildlife Research Center, Denver CO. 52 pp.

Kepler, C. B. 1967. Polynesian rat predation on nesting Laysan albatrosses and other Pacific seabirds. Auk 84:426-430.

Lindsey, G. D., and S. M. Mosher. 1994. Tests indicate minimal hazard to 'Io from diphacinone baiting. Hawaii's Forests and Wildlife 4:1,3.
Mendenhall, V. M., and L. F. Pank. 1980. Secondary poisoning of owls by anticoagulant rodenticides. Wildl. Soc. Bullet. 8:311-315.
Miller, S. E. and M. G. Hadfield. 1993. Conservation of Hawaiian tree snails. Pp. 10 in 1993 Hawaii Conservation Conference. The Hawaii Conservation Biology Initiative, Honolulu.
Mueller-Dombois, D., and C. H. Lamoureux. 1967. Soil-vegetation relationships in Hawaiian Kipukas. Pacific Science 21(2): 286-299.
Nelson, L., Jr. and F. W., Clark. 1973. Correction for sprung traps in catch/effort calculations of trapping results. J. Mammal. 54:295-298.
Perkins, R. C. L. 1903. Vertebrata. *In* D. Sharp (ed.) Fauna Hawaiiensis, Vol I, pt. IV. The University Press, Cambridge, England.
Richardson, F. and D. H. Woodside. 1954. Rediscovery of the nesting of the Dark-rumped Petrel in the Hawaiian Islands. Condor 56:323-327.
Russell, C. A. 1980. Food habits of the roof rat (Rattus rattus) in two areas of Hawaii Volcanoes National Park. Pp. 269-272 *in* C. W. Smith (ed.) Proc. 3rd Conf. In Natural Sciences, Hawaii Volcanoes National Park. Honolulu.
Savarie, P.J., D.J. Hayes R.T. McBrice, J.D. Roberts. 1979. Efficacy and safety of diphacinone as a predacide. Avian and Mammalian Wildlife Toxicology, ASTM STP 693. E.E. Kenaga, Ed., American Society for Testing and Materials, pp.69-79.
Scott, J. M., S. Mountainspring, F. L. Ramsey, and C. B. Kepler. 1986. Forest bird communities of the Hawaiian Islands; their dynamics, ecology, and conservation. Stud. Avian Biol. 9.
Scowcroft, P. G., and H. F. Sakai. 1984. Stripping of Acacia koa bark by rats on Hawaii and Maui. Pacific Science 38(1): 80-86.
Snetsinger, T. J., S. G. Fancy, J. C. Simon, and J. D. Jacobi. 1994. Diets of owls and feral cats in Hawaii. Elepaio 54:47-50.
Stone, C. P., M. Dusek, and M. Aeder. 1995. Use of anticoagulant to control mongooses in nene breeding habitat. >Elepaio 54:73-78.
Sugihara, R. T. 1997. Abundance and diets of rats in two native Hawaiian forests. *Pacific Sci.* 51:189-198.
Swift, C. E. 1998. Laboratory bioassays with wild-caught black (*Rattus rattus*) and Polynesian (*R. exulans*) to determine minimum amounts of Ramik Green (0.005% diphacinone) and exposure times for field broadcast applications in Hawaii. M.S. Thesis, Univ. Hawaii-Manoa, Honolulu. 92pp.
Tomich, P. Q. 1986. Mammals in Hawaii, 2nd ed. Bishop Museum Press, Honolulu.
Van Riper, C. III. 1980. Observations on the breeding of the Palila Psittirostra bailleui of Hawaii. Ibis 122:462-475.
Woodward, P. W. 1972. The natural history of Kure Atoll, Northwestern Hawaiian Islands. Atoll Res. Bull. 164.

XVIII. **Signature Page:**

Note: Signed copy of this document could not be located.

Study Director

Date

Study Director

Date

Approved:

Director, PIERC

Date

Appendix 2. Amendments to study protocol QA-02.

Amendment No. 1. V. Objectives.

Amendment No. 2. VII. Methods. A. Procedure. 1. Study Area.

Amendment No. 3. VII. Methods. A. Procedure. 2. Live-trapping (time intervals).

Amendment No. 4. VII. Methods. A. Procedure. 2. Live-trapping (method of analysis).

Amendment No. 5. VII. Methods. A. Procedure. 3. Census blocks.

Amendment No. 6. VII. Methods. A. Procedure. 4. Radio-telemetry.

Amendment No. 7. VII. Methods. A. Procedure. 5. Kill-trapping.

Amendment No. 8. VII. Methods. A. Procedure. 6. Diphacinone baiting.

Amendment No. 9. VII. Methods. A. Procedure. 7. Bait disappearance rate.

Amendment No. 10. VII. Methods. A. Procedure. 9. Non-target hazards (searching).

Amendment No. 11. VII. Methods. D. Experimental design and statistical analyses.

Amendment No. 12. VII. Methods. I. Analytical chemistry.

Amendment No. 13. VIII. Animal Care and Use. A. Test system.

Amendment No. 14. VIII. Animal Care and Use. M. Disposition of animals.

Amendment No. 15. XII. Schedule.

Amendment No. 1. V. Objectives.

Item to be changed:

1. Determine efficacy of hand broadcast baiting with 0.005% diphacinone bait for the control of rats in wet forest habitat.

2. Determine efficacy of hand broadcast baiting with 0.005% diphacinone bait for the control of rats in mesic forest habitat.

3. Determine efficacy of aerially broadcast baiting with 0.005% diphacinone bait.

4. Determine disappearance rate from the forest floor of diphacinone pellets.

5. Monitor secondary hazard potential of hand and aerially broadcast 0.005% diphacinone bait.

6. Monitor the impact of rodent predation on and recovery of representative populations of native flora and fauna following control applications

Revision:

1. Determine the efficacy of hand-broadcast application of 0.005% diphacinone bait for the control of rats in wet forest habitat.

2. Determine the efficacy of hand-broadcast application of 0.005% diphacinone bait for the control of rats in mesic forest habitat.

4. Determine the disappearance rate of hand-broadcast 0.005% diphacinone bait from the forest floor.

5. Monitor the secondary hazard potential of hand-broadcast 0.005% diphacinone bait.

Reasons: The study was divided into three parts (1) the efficacy and safety of hand-broadcast baiting, (2) the efficacy and safety of aerial-broadcast baiting, and (3) the impact of rodent predation on and recovery of representative populations of native flora and fauna following control applications. Each part will be reported separately.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: David Foster Date 8/25/03

QA Officer: [signature] Date 8/25/03

Amendment No. 2. VII. Methods. A. Procedure. 1. Study Area.

Item to be changed: A 25-m × 25-m grid will be established over each study area.

Revision: A 12.5-m × 12.5-m grid will be established over each study area.

Reasons: Due to the dense vegetation in the study areas, a 12.5-m × 12.5-m grid was considered necessary to ensure a more even distribution of baits during the hand-broadcast application.

Effect of Amendment: This amendment should improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 3. VII. Methods. A. Procedure. 2. Live-trapping (time intervals).

Item to be changed: Traps will also be opened 1 week, 2 months, and 4 months after the first and each subsequent hand broadcast baiting series...

Revision: Traps will also be opened approximately 3 weeks, 2 months, and when possible 4 months after each hand-broadcast baiting series...

Reasons: The intervals at which traps were opened were varied to fit in with the rate of bait acceptance by rats, weather, and other variables (such as weekends and public holidays). When the monitoring 2 months after bait application showed that the rat population had recovered to pre-treatment levels, a new application of bait was made about 1 week later, and therefore the 4-month post-application monitoring was not possible (or necessary).

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QAOfficer: _____ Date 8/25/03

Amendment No. 4. VII. Methods. A. Procedure. 2. Live-trapping (method of analysis).

Items to be changed: For each forest type (mesic and wet), rat capture rates pre- and post-treatment in the control (non-treatment) and treatment plots will be compared by Chi-square analysis.

Revision: Rat capture rates pre- and post-treatment in the control (non-treatment) and treatment plots will be compared using a generalized linear model (S-Plus for Windows, 2001, Insightful Corporation, Seattle, Washington, USA), adjusting for trap-nights by using log (trap-nights) as an offset term in the model. The ratio of the variance to the mean (a measure of dispersion) will be estimated separately for the wet forest and mesic forest, from the "plot by time" interaction in a model of the pre-treatment capture rates in the treatment and non-treatment plots (for the 12 bait applications in the wet forest and three bait applications in the mesic forest), and these values will be used to scale the residual deviances of the generalized linear model before assessing their significance against a chi-square distribution (McCullagh, P. and Nelder, J.A.,1989, Generalized Linear Models, Chapman Hall, London, UK).

Reasons: The original protocol was not reviewed by a biometrician. Normal chi-square analysis was not appropriate because (a) rat captures were over-dispersed (clustered), not independent, and chi-square analysis could not correct for this non-independence, and (b) rat captures adjusted for trap-nights could not be used as observed values because they were not integers, and their use would have resulted in expected values being less than 5. Non-independence could have resulted from factors such as rats living in pairs or family groups, rat nests being further apart than traps, and/or rat favored habitat and therefore rat nests being clustered. The generalized linear model used repeated measurements over time (the 12 pairs of pre-treatment values in wet forest and three pairs of pre-treatment values in mesic forest) to estimate the dispersion parameter.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 5. VII. Methods. A. Procedure. 3. Census blocks.

Items to be changed: Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5 m, 62.5 m, etc.) for 2 nights before baiting, and at 2 weeks, 2 months, and 4 months following each toxic baiting series ...

...the proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared by Chi-square analysis.

Revision: Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5 m, 62.5 m, etc.) for 2 nights 1–3 weeks before baiting, and at approximately 2 weeks, 2 months, and when possible 4 months following each toxic baiting series...

...the proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared by a generalized linear model (S-Plus for Windows, 2001, Insightful Corporation, Seattle, Washington, USA), with the scaled residual deviances assessed for significance against a chi-square distribution (McCullagh, P. and Nelder, J.A.,1989, Generalized Linear Models, Chapman Hall, London, UK).

Reasons: The time interval before baiting was not stated in the original protocol. The time intervals after baiting were varied from the protocol to fit in with other rat monitoring activities (e.g., live-trapping). When other monitoring 2 months after bait application showed that the rat population had recovered to pre-treatment levels, a new application of bait was made about 1 week later, and therefore the 4-month post-application monitoring was not possible (or necessary).

Normal chi-square analysis was not an appropriate method of analysis for the reasons given in Amendment No. 4 (above).

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 6. VII. Methods. A. Procedure. 4. Radio-telemetry.

Items to be changed: Before the initial bait application, 10 to 15 rats within each treatment plot and 10 to15 rats within each control plot will be fitted with transmitters.

Revision: Before the initial bait application, up to 20 rats within each study plot will be fitted with radio transmitters.

Reasons: The number of radio transmitters that could be fitted to rats depended not only on the number of transmitters available, but also on the trapping success in each study plot. In the wet forest study plots, trapping success was very low and few rats could be fitted with transmitters. In the mesic forest, trapping success was higher so it was opportunistic to fit additional rats with transmitters.

Effect of Amendment: This amendment should improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 7. VII. Methods. A. Procedure. 5. Kill-trapping.

Items to be changed: In each 4-ha study plot, 40 mouse traps (2 traps at a location) will be placed at 10 m intervals along one transect within each 4-ha study plot to estimate mouse density pre- and post-treatment. Traps will be opened 3 weeks before toxic bait is applied, 2 weeks after bait is applied, then at 2-month intervals after each hand broadcast toxic baiting series... Mouse capture rates pre-and post-treatment in the control and treatment plots will be ... compared by chi-square analysis.

Revision: In each 4-ha study plot, 42 mouse traps (2 traps at a location) will be placed at 10 m intervals along one transect line to estimate mouse density pre- and post-treatment. The traps will be opened approximately 3 weeks before, and approximately 2 weeks and 2 months after toxic bait is applied. If toxic bait is not reapplied after 2 months, traps will be opened at approximately 2-month intervals. Trapping will be stopped if too few mice or too many non-target birds are caught. Mouse capture rates pre-and post-treatment in the control and treatment plots will be ... compared by generalized linear model analysis (see amendment no. 4).

Reasons: Additional mouse traps were available, so were used to increase the sampling effort. The timing of trapping was made flexible to accommodate other monitoring activities and mouse population levels. Trapping was stopped after July 2000 because too few mice and too many non-target birds (non-native red-billed leiothrix and northern cardinals) were being caught. Mouse capture rates could not be compared by chi-square analysis for the same reasons that rat capture rates could not (see amendment no. 4).

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 8. VII. Methods. A. Procedure. 6. Diphacinone baiting.

Item to be changed: One fish-flavored formulation (Ramik® Green, HACCO, Inc., Madison, WI) of 0.005% diphacinone pellet bait (mean pellet size = 5.8 g) will be tested.

During the following one year period, hand broadcast toxic bait applications will be repeated in the treatment plots every 2–3 months or when rat populations recover to pre-treatment levels, whichever is the longer. A maximum of 5 series of toxic bait applications will be applied to each treatment plot.

Revision: One fish-flavored formulation (Ramik® Green, HACCO, Inc., Madison, WI) containing 0.005% diphacinone (mean pellet size = 5.8 g) will be tested. The mean weight, length, and width of at least one sample of bait will be measured after receipt. The mean diphacinone concentration of at least one other sample of bait will also be determined.

During the following 2-year period, hand-broadcast toxic bait applications will be repeated in the treatment plots every 2–3 months or when rat populations recover to pre-treatment levels, whichever is the longer. A maximum of 12 series of toxic bait applications will be applied to each treatment plot over the 2-year period.

Reasons: (a) The weight, length, and width, and the mean diphacinone concentration of baits were required to be assessed for quality assurance purposes.

(b) A 1-year extension to the State EUP was granted to further study the effects of controlling rat populations on seedling survival (part 3 of the amended study objectives).

Effect of Amendment: This amendment should improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 9. VII. Methods. A. Procedure. 7. Bait disappearance rate.

Item to be changed: Immediately after each toxic bait application series, the location of 10 individual baits will be marked with numbered, colored wire flags at four separate sites within each 4-ha baited area. Location of pellets will equal the density of pellets per area from actual hand broadcast distribution. Each numbered, colored wire flag will be placed at a compass bearing of 360 degrees and touching the pellet. Each pellet will be examined daily for 14 days or until eaten or the bait disintegrates.

Revision: Immediately after each toxic bait application series, 20 locations (at least 25 m apart) will be randomly selected in the first quarter of each 4-ha baited area, and the locations marked with colored wire flags. One bait will be placed beside each wire flag. Each bait will be examined at 1–3 day intervals for 14 days or until it is eaten or disintegrates.

Reasons: The protocol was amended to comply with SOP BRD-12 (Appendix 17). The number and location of baits, and the frequency of examination, was amended to fit in with the human resources available.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 10. VII. Methods. A. Procedure. 9. Non-target hazards (searching).

Item to be changed: Four transects (400 m long × 5 m wide and spaced 25 m apart; 10% of each total study plot) within each treatment and control plot will be walked to search for non-target mortality 2 weeks before, and 2 weeks and one month after each bait application series.

Revision: Four transects (400 m long × 5 m wide and spaced 25 m apart; 10% of each total study plot) within each treatment and control plot will be walked to search for non-target mortality up to 2 weeks before and 2–4 weeks after each bait application series.

Reasons: The timing of searches was varied to fit in with other monitoring activities (radio-telemetry, live-trapping, etc). As a consequence, only one search was made within 4 weeks after bait application, instead of one at 2 weeks and one at one month.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 11. VII. Methods. D. Experimental design and statistical analyses.

Item to be changed: Catch per unit effort, adjusted for sprung traps, percent census blocks gnawed, percent radio-collared rats alive, seed loss, and predation on seedlings between the control and treatment study sites in each the wet and mesic forests will be compared by chi-square analysis.

Revision: Catch per unit effort, adjusted for sprung traps, in the treatment and non-treatment plots before and after bait application will be compared by generalized linear model. Percent radio-collared rats alive and percent census blocks gnawed in the treatment and non-treatment plots before and after bait application will be compared by chi-square analysis.

Reasons: Catch per unit effort, adjusted for sprung traps, could not be analyzed by chi-square analysis (see amendment no. 4). Seed loss and predation on seedlings will be reported separately (see amendment no. 1).

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 12. VII. Methods. I. Analytical chemistry.

Item to be changed: The Ramik® Green diphacinone will be assayed for diphacinone by the U.S. Department of Agriculture, Animal & Plant Health Inspection Service, National Wildlife Research Center.

Revision: Ramik® Green baits and animal tissues will be assayed for diphacinone by Genesis Laboratories Inc. (Wellington, CO) or Landcare Research (Lincoln, New Zealand).

Reasons: (a) Animal tissues as well as baits needed to be analyzed. (b) The National Wildlife Research Center was unable to do the analyses. The alternative laboratories, Genesis and Landcare Research, are both GLP accredited. The final choice of laboratory was dictated by cost-effectiveness.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 13. VIII. Animal Care and Use. A. Test system.

Item to be changed: Mice live captured will be immediately released.

Revision: Mice captured alive will be immediately released or euthanazed using CO_2.

Reasons: Some mice captured alive were required for analysis of diphacinone residues for another study.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 14. VIII. Animal Care and Use. M. Disposition of animals.

Item to be changed: All recovered animals will be retained at USGS/BRD Pacific Island Ecosystems Research Center's Kilauea Field Station or disposed of by burial at the study site.

Revision: All recovered animals will be (a) retained at USGS/BRD Pacific Island Ecosystems Research Center's Kilauea Field Station, (b) sent to a laboratory for analysis of diphacinone residues, or (c) disposed of by burial at the study site.

Reasons: Some recovered animals were required to be analyzed for diphacinone residues.

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 15. XII. Schedule.

Item to be changed:

Proposed Experiment Start Date:	Oct 1999
Proposed Experiment Termination Date:	Oct 2001
Proposed Study Completion Date:	July 2002

Revision:

Proposed Experiment Start Date:	Oct 1999
Proposed Experiment Termination Date:	Dec 2001
Proposed Study Completion Date:	Dec 2002

Reasons: Two 1-year extensions to the State EUP were granted to further study the effects of controlling rat populations on seedling survival (part 3 of the amended study objectives) (see Appendix 2, amendment 1, and Appendix 4).

Effect of Amendment: This amendment should not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Appendix 3. Deviations to study protocol QA-02.

Deviation No. 1. VII. Methods. A. Procedure. 2. Live-trapping.

Deviation No. 2. VII. Methods. A. Procedure. 3. Census blocks.

Deviation No. 3. VII. Methods. A. Procedure. 4. Radio-telemetry (nest locations).

Deviation No. 4. VII. Methods. A. Procedure. 4. Radio-telemetry (method of analysis).

Deviation No. 5. VII. Methods. A. Procedure. 5. Kill-trapping (days set).

Deviation No. 6. VII. Methods. A. Procedure. 5. Kill-trapping (methods of analysis).

Deviation No. 7. VII. Methods. A. Procedure. 7. Bait disappearance rate.

Deviation No. 8. VII. Methods. A. Procedure. 8. Environmental conditions.

Deviation No. 9. VII. Methods. A. Procedure. 9. Non-target hazards (searches for dead non-target species).

Deviation No. 10. VII. Methods. A. Procedure. 9. Non-target hazards (collection of birds).

Deviation No. 1. VII. Methods. A. Procedure. 2. Live-trapping.

Protocol deviated: All rats captured will be identified to species, sexed, aged, weighed, ear-tagged and released.

Deviation: Not all rats captured were identified to species, sexed, weighed, or ear-tagged.

Reasons: Some rats escaped before they could be identified to species, sexed, weighed, or ear-tagged.

Effect of Deviation: This deviation meant that some information about the species, sex, weight, and capture status of individual rats was lost, but it did not affect the main outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 2. VII. Methods. A. Procedure. 3. Census blocks.

Protocol deviated: Blocks will be placed on transects ... for 2 nights before baiting, and at 2 weeks, 2 months, and 4 months following each toxic baiting series to determine efficacy and rat reinvasion rates ... For each forest type (mesic and wet), the proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared ...

Deviation: The proportion of census blocks gnawed on by rats pre- and post-treatment in the control and treatment plots could not be compared for the 18 January bait application in the wet forest.

Reasons: The data from the treatment plot before baiting were lost.

Effect of Deviation: This deviation meant that some information about the species, sex, weight, and capture status of individual rats was lost, but it did not affect the main outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 3. VII. Methods. A. Procedure. 4. Radio-telemetry (nest locations).

Protocol deviated: Nest locations will be recorded, nest material identified, and the nest will be measured (width, depth, inside diameter). Nests will be collected by placement in a plastic ZipLok bag. Woody trees and tree ferns containing rat nests will be identified, measured (trunk diameter at 4.5'), and the height of the nest in the tree determined.

Deviation: Nest material was not always identified, the nest size not always measured, nests not always collected, and height of the nest in the tree not always determined.

Reasons: Some nests were too high and/or too difficult to get to.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 4. VII. Methods. A. Procedure. 4. Radio-telemetry (method of analysis).

Protocol deviated: For each forest type (mesic and wet), the proportion of radio-tagged rats surviving in post-treatment will be compared by Chi-square analysis.

Deviation: The proportion of radio-tagged rats surviving after bait application in the wet forest could not be compared by Chi-square analysis.

Reasons: Too few rats were captured and radio-tagged.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 5. VII. Methods. A. Procedure. 5. Kill-trapping (days set).

Protocol deviated: During each trapping session, traps will be baited with coconut chunks, opened, and examined daily for captures for 2 days.

Deviation: In the June 2000 pre-treatment trapping session in the wet forest, traps were opened for only 1 day.

Reasons: Staff was not available for the second day.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 6. VII. Methods. A. Procedure. 5. Kill-trapping (methods of analysis).

Protocol deviated: Mouse capture rates pre- and post-treatment in the control and treatment plots will be ... compared by chi-square analysis.

Deviation: Mouse capture rates could not be compared statistically.

Reasons: Too few mice were caught.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 7. VII. Methods. A. Procedure. 7. Bait disappearance rate.

Protocol deviated: Each pellet will be examined daily for 14 days or until eaten or the bait disintegrates. Data will be recorded on disappearance, consumption by rats, and feeding by slugs, snails and other invertebrates.

Deviation: Data on feeding by slugs, snails and other invertebrates was not always recorded.

Reasons: It was not always possible to distinguish accurately between feeding by rats, mice, and different species of invertebrates.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 8. VII. Methods. A. Procedure. 8. Environmental conditions.

Protocol deviated: Daily rainfall and temperature will be recorded by 10 a.m. each morning for 2 weeks after each bait distribution series. Rainfall will be recorded at weekly intervals between bait applications.

Deviation: Daily rainfall and temperature were not always recorded daily during the 2 weeks after each bait application series, and rainfall was not always recorded weekly between bait applications.

Reasons: The original study protocol was too ambitious for the human resources available. It was not feasible to visit the study sites daily for 2 weeks after each bait distribution series, or weekly between bait applications, with the resources available.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 9. VII. Methods. A. Procedure. 9. Non-target hazards (searches for dead non-target species).

Protocol deviated: Four transects (400 m long × 5 m wide and spaced 25 m apart; 10% of each total study plot) within each treatment and control plot will be walked to search for non-target mortality 2 weeks before, and 2 weeks and one month after each bait application series…

Deviation: Transects in the wet forest were not searched after the January 2001 bait application, nor before or after the May, August, and December 2001 bait applications.

Reasons: Staff was not available (January 2001) and no non-target mortality had been found on previous searches.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 10. VII. Methods. A. Procedure. 9. Non-target hazards (collection of birds).

Protocol deviated: Birds will be collected using mist nets or shooting (kalij pheasant) in each treatment plot 1–4 weeks after baiting.

Deviation: Birds were collected for more than 6 weeks after the first bait application series. Birds found dead in live-traps (for rats) or kill-traps (for mice) were also collected.

Reasons: The period of collection was extended to increase sample sizes, because too few birds were caught within the original timeframe. With the resources available, it was possible to purposefully collect samples only after the first bait application series, not after each bait application series. Birds found dead in live-traps and kill-traps were also collected to increase sample sizes.

Effect of Deviation: This deviation did not affect the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Appendix 4. Experimental Use Permits (EUP-99-01 and EUP-99-02).

w. 10/95

R Boesch

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION-PESTICIDES

APPLICANT

a. NAME OF APPLICANT: Gerald D. Lindsey
c. OF APPLICANT: Wildlife Biologist
d. TELEPHONE: 808-967-7396 ext 232

b. COMPANY NAME & ADDRESS: USGS- Biological Resources Division, Pacific Island Ecosystems Research Center, P.O. Box 44, Hawaii National Park, HI 96718

PESTICIDE

a. BRAND NAME (if any): Ramik Green
b. EPA Registration Number or other I.D. Number: 2393-498
c. ACTIVE INGREDIENTS (by chemical name): Diphacinone
d. LICENSED IN HAWAII? [X] YES [ ] NO

DESCRIPTION OF EXPERIMENT (Submit Copy of Experimental Protocol)

a. LOCATION OF TRIAL(s) (AREA, TOWN OR CITY, FIELD NO., AND ISLAND): Hawaii Volcanoes National Park, Olaa Forest Reserve, Koa Unit, Island of Hawaii

b. SIZE OF TRIALS (acres, sq. ft., etc.): 10 acres
c. NUMBER OF TRIALS: One
d. NUMBER OF REPLICATIONS: 0

e. COMMODITY (crop) TO BE TREATED: Wet Forest (Ohia trees Metrosideros polymorpha and tree ferns Cibotium spp.
f. STAGE OF GROWTH OF COMMODITY: N/A

g. PEST(s): Rat (Rattus spp.)

h. DOSAGE RATE(s) (lbs. active per unit area): 0.907g to 2.72g/acre (0.032 oz to 0.096 oz/acre)
i. METHOD OF APPLICATION: [X] GROUND [ ] AERIAL [ ] OTHER (specify)

DURATION OF EXPERIMENT

a. STARTING DATE: July 1999
b. COMPLETION DATE: Dec. 2000

TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in Metrosideros/Cibotium wet forest for the protection of endemic wildlife and plants.

DISPOSITION OF TREATED COMMODITY: N/A

PERSON SUPERVISING TRIAL

NAME OF PERSON: Gerald D. Lindsey
TITLE OF PERSON: Wildlife Biologist
TELEPHONE: 808-967-7396 ext 232
e. CATEGORY 10 CERT. NO.: H71592

b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

SIGNATURE OF PERSON: Gerald D Lindsey
g. DATE: April 20, 1999

FOR STATE USE ONLY

DISPOSITION: [X] APPROVED [ ] DISAPPROVED
STATE EUP NO.: EUP-99-01
EXPIRATION DATE: December 31, 2000

RESTRICTIONS

1) All applications shall be made by or under the direct supervision of Mr. Gerald D. Lindsey (H71592). 2) Applications shall be made in accordance with the attached study protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) The Department of Agriculture, Pesticides Branch Office in Hilo (974-4143) shall be notified of the initial application to allow inspectors an opportunity to monitor the application. 4) All data must be gathered following procedures comparable to that of subpart G - Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 5) Adverse effects resulting from this use shall be reported to the Department of Agriculture immediately at (808) 973-9401. 6) A report summarizing the results of this use shall be submitted to the Department of Agriculture within 6 months following the conclusion of this EUP. 7) All applicable restrictions appearing on the section 3 (container) label shall be followed.

QA-02

NAME AND TITLE OF STATE OFFICIAL	SIGNATURE	DATE
Alice K. Kobashigawa, Pesticide Specialist	Alice K Kobashigawa	5/05/99

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION-PESTICIDES

APPLICANT

a. NAME OF APPLICANT: Gerald D. Lindsey

c. TITLE OF APPLICANT: Wildlife Biologist

d. PHONE: 808-967-7396 ext 232

b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

PESTICIDE

a. BRAND NAME (if any): Ramik Green

b. EPA Registration Number or other I.D. Number: 2393-498

c. ACTIVE INGREDIENTS (by chemical name): Diphacinone

d. LICENSED IN HAWAII? [X] YES [ ] NO

DESCRIPTION OF EXPERIMENT (Submit Copy of Experimental Protocol)

a. LOCATION OF TRIAL(s) (AREA, TOWN OR CITY, FIELD NO., AND ISLAND): Hawaii Volcanoes National Park, Kipuka Ki, Island of Hawaii

a. SIZE OF TRIALS (acres, sq. ft., etc.): 10 acres

c. NUMBER OF TRIALS: one

d. NUMBER OF REPLICATIONS: 0

e. COMMODITY (crop) TO BE TREATED: Mesic Forest (koa/ohia/soap berry trees)

f. STAGE OF GROWTH OF COMMODITY: N/A

g. PEST(s): Rat (Rattus spp.)

h. DOSAGE RATE(s) (lbs. active per unit area): 0.907g to 2.7g per acre (0.032 oz to 0.096 oz per acre)

i. METHOD OF APPLICATION: [X] GROUND [ ] AERIAL [ ] OTHER (specify)

DURATION OF EXPERIMENT

a. STARTING DATE: July 1999

b. COMPLETION DATE: Dec 2000

j. TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in mesic forest for the protection of endemic wildlife and plants

k. DISPOSITION OF TREATED COMMODITY: N/A

PERSON SUPERVISING TRIAL

a. NAME OF PERSON: Gerald D. Lindsey

TITLE OF PERSON: Wildlife Biologist

TELEPHONE: 808-967-7396 ext 232

c. CATEGORY/CERT. NO.: H71592

b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

SIGNATURE OF PERSON: Gerald D Lindsey

g. DATE: April 20, 1999

FOR STATE USE ONLY

DISPOSITION: [X] APPROVED [ ] DISAPPROVED

STATE EUP NO.: EUP-99-02

EXPIRATION DATE: December 31, 2000

RESTRICTIONS

1) All applications shall be made by or under the direct supervision of Mr. Gerald D. Lindsey (H71592). 2) Applications shall be made in accordance with the attached study protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) The Department of Agriculture, Pesticides Branch Office in Hilo (Ph. 974-4143) shall be notified of the initial application to allow inspectors an opportunity to monitor the application. 4) All data must be gathered following procedures comparable to that of subpart G - Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 5) Adverse effects resulting from this use shall be reported to the Department of Agriculture immediately at (808) 973-9401. 6) A report summarizing the results of this use shall be submitted to the Department of Agriculture within 6 months following the conclusion of this EUP. 7) All applicable restrictions appearing on the section 3 ntainer) label shall be followed.

QA-02

NAME AND TITLE OF STATE OFFICIAL	SIGNATURE	DATE
Lance K. Kobashigawa, Pesticide Spec.	Lance K. Kobashigawa	5/05/99

BENJAMIN J. CAYETANO
Governor

JAMES J. NAKATANI
Chairperson, Board of Agriculture

LETITIA UYEHARA
Deputy to the Chairperson

State of Hawaii
DEPARTMENT OF AGRICULTURE
Pesticides Branch
1481 South King Street, Suite 431
Honolulu, Hawaii 96814

Mailing Address:
P.O. Box 22159
Honolulu, Hawaii 96823-2159

FAX: (808) 973-9418

March 9, 2000

Gerald D. Lindsey, Wildlife Biologist
U.S. Geological Survey
Pacific Island Ecosystems Research Center
Kilauea Field Station
P. O. Box 44, Building 344
Hawaii National Park, HI 96718

Dear Gerald:

SUBJECT: State EUP Numbers EUP-99-01 and EUP-99-02; Your March 7, 2000 Letter Requesting for a 1-Year Extension

Your request to amend the current expiration date of December 31, 2000 to December 31, 2001 is hereby approved. Copies of the amended certificates are enclosed for your files.

Should you have any questions, please contact me at (808) 973-9415.

Sincerely,

Lance K. Kobashigawa
Pesticide Specialist

[glindsey]

c: GSahara, Enforcement - Hilo

QA-02

Rev 10/95

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION-PESTICIDES

1. APPLICANT

a. NAME OF APPLICANT: Gerald D. Lindsey

c. TITLE OF APPLICANT: Wildlife Biologist

d. TELEPHONE: 808-967-7396 ext 232

b. COMPANY NAME & ADDRESS: USGS- Biological Resources Division
Pacific Island Ecosystems Research Cent
P.O. Box 44
Hawaii National Park, HI 96718

2. PESTICIDE

a. BRAND NAME (if any): Ramik Green

b. EPA Registration Number or other I.D. Number: 2393-498

c. ACTIVE INGREDIENTS (by chemical name): Diphacinone

d. LICENSED IN HAWAII? [X] YES [ ] NO

3. DESCRIPTION OF EXPERIMENT (Submit Copy of Experimental Protocol)

a. LOCATION OF TRIAL(s) (AREA, TOWN OR CITY, FIELD NO., AND ISLAND): Hawaii Volcanoes National Park, Olaa Forest Reserv
Koa Unit, Island of Hawaii

b. SIZE OF TRIALS (acres, sq. ft., etc.): 10 acres

c. NUMBER OF TRIALS: One

d. NUMBER OF REPLICATIONS: 0

c. COMMODITY (crop) TO BE TREATED: Wet Forest (Ohia trees Metrosideros polymorpha and tree ferns Cibctium spp.

f. STAGE OF GROWTH OF COMMODITY: N/A

g. PEST(s): Rat (Rattus spp.)

h. DOSAGE RATE(s) (lbs. active per unit area): 0.907g to 2.72g/acre (0.032 oz to 0.096 oz/acre)

i. METHOD OF APPLICATION [X] GROUND [ ] AERIAL [ ] OTHER (specify)

4. DURATION OF EXPERIMENT

a. STARTING DATE: July 1999

b. COMPLETION DATE: Dec. 2000

5. TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in Metrosideros/Cibotium wet forest for the protection of endemic wildlife and plants.

6. DISPOSITION OF TREATED COMMODITY: N/A

7. PERSON SUPERVISING TRIAL

a. NAME OF PERSON: Gerald D. Lindsey

c. TITLE OF PERSON: Wildlife Biologist

d. TELEPHONE: 808-967-7396 ext 232

e. CATEGORY 10 CERT. NO.: H71592 EXP. 12/16/2001

b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division
Pacific Island Ecosystems Research Center
Kilauea Field Station, P.O. Box 44
Hawaii National Park, HI 96718

f. SIGNATURE OF PERSON: Gerald D Lindsey

g. DATE: April 20, 1999

FOR STATE USE ONLY

DISPOSITION: [X] APPROVED [ ] DISAPPROVED

STATE EUP NO.: EUP-99-01

EXPIRATION DATE: December 31, ~~2000~~ 2001

RESTRICTIONS

1) All applications shall be made by or under the direct supervision of Mr. Gerald D. Lindsey (H71592). 2) Applications shall be made in accordance with the attached study protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) The Department of Agriculture, Pesticides Branch Office in Hilo (974-4143) shall be notified of the initial application to allow inspectors an opportunity to monitor the application. 4) All data must be gathered following procedures comparable to that of subpart G - Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 5) Adverse effects resulting from this use shall be reported to the Department of Agriculture immediately at (808) 973-9401. 6) A report summarizing the results of this use shall be submitted to the Department of Agriculture within 6 months following the conclusion of this EUP. 7) All applicable restrictions appearing on the section 3 (container) label shall be followed.

QA-02

NAME AND TITLE OF STATE OFFICIAL: Lance K. Kobashigawa, Pesticide Specialist

SIGNATURE: Lance K Kobashigawa

DATE: 5/05/99

Rev. 10/95

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION-PESTICIDES

1. APPLICANT

a. NAME OF APPLICANT: Gerald D. Lindsey
c. TITLE OF APPLICANT: Wildlife Biologist
d. TELEPHONE: 808-967-7396 ext 232
b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

2. PESTICIDE

a. BRAND NAME (if any): Ramik Green
b. EPA Registration Number or other I.D. Number: 2393-498
c. ACTIVE INGREDIENTS (by chemical name): Diphacinone
d. LICENSED IN HAWAII? [X] YES [ ] NO

3. DESCRIPTION OF EXPERIMENT (Submit Copy of Experimental Protocol)

a. LOCATION OF TRIAL(s) (AREA, TOWN OR CITY, FIELD NO., AND ISLAND): Hawaii Volcanoes National Park, Kipuka Ki, Island of Hawaii
b. SIZE OF TRIALS (acres, sq. ft., etc.): 10 acres
c. NUMBER OF TRIALS: one
d. NUMBER OF REPLICATIONS: 0
e. COMMODITY (crop) TO BE TREATED: Mesic Forest (koa/ohia/soapberry trees)
f. STAGE OF GROWTH OF COMMODITY: N/A
g. PEST(s): Rat (Rattus spp.)
h. DOSAGE RATE(s) (lbs. active per unit area): 0.907g to 2.7g per acre (0.032 oz to 0.096 oz per acre)
i. METHOD OF APPLICATION: [X] GROUND [ ] AERIAL [ ] OTHER (specify)

4. DURATION OF EXPERIMENT

a. STARTING DATE: July 1999
b. COMPLETION DATE: Dec. 2000

5. TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in mesic forest for the protection of endemic wildlife and plants

6. DISPOSITION OF TREATED COMMODITY: N/A

7. PERSON SUPERVISING TRIAL

a. NAME OF PERSON: Gerald D. Lindsey
c. TITLE OF PERSON: Wildlife Biologist
d. TELEPHONE: 808-967-7396 ext 232
e. CATEGORY/10 CERT. NO.: H71592
b. COMPANY NAME & ADDRESS: USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718
f. SIGNATURE OF PERSON: Gerald D Lindsey
g. DATE: April 20, 1999

FOR STATE USE ONLY

DISPOSITION: [X] APPROVED [ ] DISAPPROVED
STATE EUP NO.: EUP-99-02
EXPIRATION DATE: December 31, ~~2000~~ 2001

RESTRICTIONS

1) All applications shall be made by or under the direct supervision of Mr. Gerald D. Lindsey (H71592). 2) Applications shall be made in accordance with the attached study protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) The Department of Agriculture, Pesticides Branch Office in Hilo (Ph. 974-4143) shall be notified of the initial application to allow inspectors an opportunity to monitor the application. 4) All data must be gathered following procedures comparable to that of subpart G - Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 5) Adverse effects resulting from this use shall be reported to the Department of Agriculture immediately at (808) 973-401. 6) A report summarizing the results of this use shall be submitted to the Department of Agriculture within 6 months following the conclusion of this EUP. 7) All applicable restrictions appearing on the section 3 (container) label shall be followed.

QA-02

NAME AND TITLE OF STATE OFFICIAL: Lance K. Kobashigawa, Pesticide Spec.
SIGNATURE: Lance K. Kobashigawa
DATE: 5/05/99

Hawaii Department of Agriculture

Pesticides Branch
1481 S. King Street, Rm. 431
Honolulu, HI 96814
Phone: (808) 973-9415
FAX (808) 973-9418

facsimile transmittal

To:	David Foote, Ph.D. Ecologist	**Fax:**	(808) 967-7153
From:	Lance Kobashigawa Pesticide Specialist	**Date:**	4/2/2002
Re:	EUP-99-01 and EUP-99-02	**Pages:**	3 (Including cover)
CC:	[Click here and type name]		

☐ Urgent ☐ For Review ☐ Please Comment ☐ Please Reply X For Your Information

Hard copy to follow in mail.

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION – PESTICIDES

1. APPLICANT

a. Name of Applicant: David Foote

b. Company Name and Address: USGS-BRD-Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

c. Title of Applicant: Ecologist

d. Telephone No.: (808) 985-6071

e. Fax No.: 808-967-7153

2. PESTICIDE

a. Brand Name (if any): Ramik Green

b. EPA Registration Number or other I.D. number: 2393-498

c. Active Ingredient(s) (by chemical name): Diphacinone

d. Is Product Licensed in Hawaii? [X] Yes [ ] No

3. DESCRIPTION OF EXPERIMENT (submit copy of experimental protocol)

a. Location of Trial(s) (Area, Town or City, Field No., and Island): Olaa Rainforest, Hawaii National Park, Koa unit, Island of Hawaii

b. Size of Trials (acres, sq. ft., etc.): 10 acres

c. Number of Trials: one

d. Number of Replications: 9

e. Commodity (crop) to be Treated: Wet forest (Ohia trees/tree ferns)

f. Stage of Growth of Commodity: N/A

g. Pest(s): Rat (Rattus spp.)

h. Dosage Rate(s) (lbs. active ingredient per unit area): 0.907g to 2.72g/acre, 0.032 oz to 0.096 oz/acre

i. Method of Application: [X] Ground [ ] Aerial [ ] Other (specify):

APR 1 2002

4. DURATION OF EXPERIMENT

a. Starting Date: July 1999

b. Completion Date: Dec 2002

5. TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in Ohia/tree fern wet forest for the protection of endemic wildlife + plants.

6. DISPOSITION OF TREATED COMMODITY: N/A

7. PERSON SUPERVISING TRIALS

a. Name of Person: Charlotte Forbes Perry

b. Company Name and Address: USGS – BRD – Kilauea Field Station, P. O. Box 44, Hawaiian National Park, HI 96718

c. Title of Person: Wildlife Biologist

d. Telephone: 808-967-7396 x240

e. Category 10 Certification No.: H71715

f. Signature: Charlotte M. Forbes Perry

g. Date: 2/22/02

FOR STATE USE ONLY

Disposition: [X] Approved [ ] Disapproved

State EUP No.: EUP-99-01

Expiration Date: December 31, 2002

RESTRICTIONS

*** This EUP is an extension of EUP-99-01 – This extension does not permit treatment of "new" acreages. Total acreage treated over the entirety of this EUP shall not exceed 10 acres.***

1) All applications shall be made under the direct supervision of Charlotte Forbes Perry (H71715). 2) All applications shall be made in accordance with the original experimental protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) All data must be gathered following procedures comparable to that of subpart G – Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 4) Any adverse effects resulting from this use shall be reported to the Department of Agriculture immediately (808) 973-9401 (Honolulu) or (808) 974-4143 (Hilo). 5) A report summarizing this use shall be submitted to the Department of Agriculture within six (6) months following the expiration of the EUP. 6) All applicable restrictions appearing on the section 3 (container) label shall be followed.

Name & Title of State Official	Signature	Date
Lance Kobashigawa, Pesticide Specialist	Lance Kobashigawa	April 2, 2002

HAWAII DEPARTMENT OF AGRICULTURE EXPERIMENTAL USE PERMIT APPLICATION – PESTICIDES

1. APPLICANT

a. Name of Applicant: David Foote
c. Title of Applicant: Ecologist
d. Telephone No.: 808-985-6071
e. Fax No.: 808-967-7153
b. Company Name and Address: USGS - BRD - Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718

2. PESTICIDE

a. Brand Name (if any): Ramik Green
b. EPA Registration Number or other I.D. number: 2393-498
c. Active Ingredient(s) (by chemical name): Diphacinone
d. Is Product Licensed in Hawaii? [X] Yes [ ] No

3. DESCRIPTION OF EXPERIMENT (submit copy of experimental protocol)

a. Location of Trial(s) (Area, Town or City, Field No., and Island): Mesic forest, Kipuka Ki, Hawaii Volcanoes N.P, Island of Hawaii
b. Size of Trials (acres, sq. ft., etc.): 10acres
c. Number of Trials: one
d. Number of Replications: 0
e. Commodity (crop) to be Treated: Mesic forest, (Koa, ohia, soapberry trees)
f. Stage of Growth of Commodity: N/A
g. Pest(s): Rat (Rattus spp)
h. Dosage Rate(s) (lbs. active ingredient per unit area): 0.907g to 2.72g /acre, 0.032oz to 0.0960z /acre
i. Method of Application: [X] Ground [ ] Aerial [ ] Other (specify):

4. DURATION OF EXPERIMENT

a. Starting Date: July 1999
b. Completion Date: Dec 2002

5. TYPE OF DATA SOUGHT: Percent reduction (control) of rat population in mesic forest for the protection of endemic wildlife and plants.

6. DISPOSITION OF TREATED COMMODITY: N/A

7. PERSON SUPERVISING TRIALS

a. Name of Person: Charlotte Forbes Perry
c. Title of Person: Wildlife Biologist
d. Telephone: 808-967-7396 x240
e. Category 10 Certification No.: H71715
b. Company Name and Address: USGS - BRD - Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718
f. Signature: Charlotte M. Forbes Perry
g. Date: 2/22/02

FOR STATE USE ONLY

Disposition: [X] Approved [ ] Disapproved
State EUP No.: EUP-99-02
Expiration Date: December 31, 2002

RESTRICTIONS

*** This EUP is an extension of EUP-99-02 – This extension does not permit treatment of "new" acreages. Total acreage treated over the entirety of this EUP shall not exceed 10 acres.***

1) All applications shall be made under the direct supervision of Charlotte Forbes Perry (H71715). 2) All applications shall be made in accordance with the original experimental protocol entitled "Testing small mammal toxicants and application methods in Hawaiian mesic and wet forests". 3) All data must be gathered following procedures comparable to that of subpart G – Protocol for and Conduct of a Study, Part 160, Good Laboratory Practices Standard. 4) Any adverse effects resulting from this use shall be reported to the Department of Agriculture immediately (808) 973-9401 (Honolulu) or (808) 974-4143 (Hilo). 5) A report summarizing this use shall be submitted to the Department of Agriculture within six (6) months following the expiration of the EUP. 6) All applicable restrictions appearing on the section 3 (container) label shall be followed.

Name & Title of State Official	Signature	Date
Lance Kobashigawa, Pesticide Specialist	Lance Kobashigawa	April 2, 2002

Appendix 5. Concurrence letter from U.S. National Park Service.

United States Department of the Interior

NATIONAL PARK SERVICE
Hawaii Volcanoes National Park
P. O. Box 52
Hawai'i 96718-0052
808/985-6000
808/967-8186 (FAX)

In Reply Refer to:

L7617(HAVO)

March 16, 2000

Dr. Gerald Lindsey
U.S. Geological Survey/Biological Resources Division
P.O. Box 44
Hawai'i National Park, Hawai'i 96718

Dear Dr. Lindsey:

The National Park Service, Hawai'i Volcanoes National Park, strongly supports research on Ramik Green pelletized pesticide in the national park, and, if registration is achieved, application and use of this pesticide in management programs. Rats are a serious threat to the survival of threatened and endangered plants and animals and are probably significant modifiers of native communities. Currently registered control technology with bait stations is prohibitively labor intensive and not suitable for application in remote sites. We anticipate that aerially broadcast pellets of Ramik Green will result in significant recovery of many native plant and animal species and help recover threatened and endangered species. The National Park Service, along with other conservation agencies in Hawai'i, have long identified aerial broadcast toxicants as an important, needed management tool. I estimate that an effective broadcast bait would be used in over 6,000 acres of the Park, once registration is achieved.

Hawai'i Volcanoes National Park's interest in management uses of Ramik Green is shown by its strong support of the current research effort by the Biological Resources Division of USGS (BRD). I prepared an Environmental Assessment (EA) for the current research program. A Finding of No Significant Impact was signed by the Regional Director. The Park sponsored a proposal to fund research on Ramik Green in the Park, and is cooperating with other investigators on studies on non-target organisms. Research by the Biological Resources Division of USGS began in the Park in October, 1999 in a rain forest site and early this year in a mesic forest site. Since then the Park has allocated funding from the fees revenues to test pesticide residues in non-target organisms.

Sincerely,

Tim Tunison
Chief of Resources Management

Appendix 6. Concurrence letter from U.S. Fish and Wildlife Service.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
HAKALAU FOREST NATIONAL WILDLIFE REFUGE
32 Kinoole Street, Suite 101
Hilo, Hawaii 96720

April 14, 2000

Dr. Gerald Lindsey
US Geological Survey/Biological Resources Division
Kilauea Field Station
P.O. Box 44
Hawaii National Park, Hawaii 96718

Dear Gerald,

The Big Island National Wildlife Refuge Complex, Hakalau Forest National Wildlife Refuge, is comprised of two units, the Hakalau Forest Unit and the Kona Forest Unit, totaling over 38,000 acres. This refuge was established to protect, preserve and enhance endangered forest birds, plants and their native forest habitat. Three species of introduced rats are found at the refuge. They are a serious threat to endemic flora and fauna and may also contribute significantly to the modification and reduction of their habitat. Hakalau Forest NWR is home to at least 22 endangered species. Over the past 5 years, field biologists and researchers at Hakalau Forest NWR have shown that rat predation is one the main causes of nest failure for native forest birds. Rats also cause fruit and seed herbivory and mortality on endangered plants found at the refuge. Research at the refuge by USGS-BRD has shown that rat control, using rodenticides in bait stations, will lessen or alleviate these problems. Currently, the refuge deploys bait stations selectively to protect vulnerable endangered plants during fruiting. If registration can be achieved and funding approved, the estimated use of aerial broadcast of rodenticides at the refuge would be approximately 7,500 acres. The cost of using the bait station methodology over an area this large in both man hours and dollars is prohibitive, and because of this, funding has yet to be targeted for rat control.

New Zealand's conservation programs, using aerial broadcast rodenticides to reduce avian predators and eliminate native plant herbivory by alien species, have been very successful. Conservation agencies in Hawaii need an efficient, low cost means of rat control to help save native species and protect the resources we are mandated to protect. Aerial broadcast of rodenticides will add an effective resource management tool. Of course, the need for testing aerial broadcast bait, its efficacy and its effects on non-target species is important before full scale applications can be considered. The Big Island National Wildlife Refuge Complex fully supports the ongoing studies on Ramik Green by HVNP and USGS-BRD and once registration of the aerial broadcast rodenticides is achieved, supports the use in management programs.

Sincerely,

Richard C Wass

Richard C Wass
Refuge Manager

QA-02

Appendix 7. Concurrence letter from State of Hawaii Division of Forestry and Wildlife.

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
DIVISION OF FORESTRY AND WILDLIFE
1151 PUNCHBOWL STREET
HONOLULU, HAWAII 96813

17 April, 2000

Gerald D. Lindsey
U.S. Geological Survey
Biological Resources Division
Kilauea Field Station
P. O. Box 44
Hawaii National Park, HI 96718

Dear Gerald,

This letter is in support of your application to receive an experimental use permit to test aerial application of diphacinone bait in Hawaiian forests for rat control. As you know, the onslaught of invasive alien species is the pre-eminent conservation problem in Hawaii. Of the 200-500 estimated alien species that are serious disruptors of native Hawaiian ecosystems, among the most pervasive and damaging for a host of native species are the three species of rat. One or another of these species occupies virtually all native habitats within the state, often at high population densities. Their documented impacts include girdling of native trees and shrubs, cessation of endangered plant recruitment by complete consumption of seed production, predation on endangered tree snails, and predation on native bird eggs and nestlings. In many instances, rat predation has been documented to be among the most important factors leading to population declines in a diversity of endangered native species.

Given that our Department manages over 800,000 acres of land across the state, including over 100,000 acres of natural area reserves preserved for their unique natural resources, it is obvious that rat control via bait stations is impossible for any but the smallest management units. In order to recover our many endangered species and protect our forests from further rat damage, the need is great for a registration to aerially apply rat toxicants in Hawaii. To that end, we view your continued research on rat toxicant efficacy, and receipt of an experimental use permit, as a critical component in achieving this registration to meet our management needs. You have our continued support and gratitude for your efforts on behalf of this goal.

Sincerely,

Michael G. Buck
Administrator

QA-02

Appendix 8. Concurrence letter from The Nature Conservancy of Hawaii.

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f Hawai'i
anu Avenue
u, Hawai'i 96817
hone (808) 537-4508
csimile (808) 545-2019

The Nature Conservancy of Hawai'i

The Nature Conservancy®
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5 N. Fairfax Dr., Suite 100
ngton, VA 22203-1606
p:// www.tnc.org

16 March 2000

Gerald D. Lindsey
U.S. Geological Survey
Biological Resources Division
Kilauea Field Station
P. O. Box 44
Hawaii National Park, HI 96718

Dear Gerald,

The Nature Conservancy of Hawaii (TNCH) manages ten preserves, encompassing over 25,000 acres, in the Hawaiian Islands. Through partnerships with federal and state government agencies and private landowners, TNCH also assists with management of other natural areas in the state. As land managers, TNCH is acutely aware of the problems posed by rats. None of the 3 species of rats (black, Polynesian, and Norway) is native to Hawaii. Rats eat the increasingly rare native snails as well as native birds, their eggs and chicks. Rats also eat flowers, fruits and seedlings of various native plants. They are also likely predators on native arthropods. TNCH staff from all islands have reported observations of rat damage to native organisms.

Currently TNCH deploys rat bait selectively to protect certain native plants and animals. However, bait must be placed in boxes that must be checked and refilled regularly. Many of the remaining natural areas in Hawaii are located in steep terrain, accessible only by helicopter at great expense. Deploying and checking bait boxes in these thickly vegetated remote areas is not cost-effective. Yet this is where many of Hawaii's remaining native species live. A low-cost and effective rat bait delivery system for these areas is crucial.

TNCH recognizes the need for thorough testing and study of aerial rat baiting. Questions about the efficacy of aerial bait broadcast and potential secondary or non-target effects must be addressed. The proposed study by the USGS-Biological Resources Division is an important step in determining the appropriateness of this technique. Although TNCH would consider aerial application of rat bait, were it to be approved, the use would be contingent on approval from the communities and partners with whom we work. Such a decision would also be based on specific threat and resource conditions within each preserve area. Scientific data from a study such as this is important for

making reasonable decisions about rat control in natural areas. The Nature Conservancy of Hawaii strongly supports this research and looks forward to the results of this project.

Sincerely,

Coleen Cory

Coleen Cory, Ph.D.
Stewardship Ecologist
The Nature Conservancy of Hawaii

Appendix 9. Biological opinion from U.S. Fish and Wildlife Service.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Pacific Islands Ecoregion
300 Ala Moana Boulevard, Room 3-122
Box 50088
Honolulu, Hawaii 96850

AUG - 6 1999

In Reply Refer To: 1-2-99-F-03; MSR

MEMORANDUM

To: Tim Tunison, Hawaii Volcanoes National Park Volcano, Hawaii

From: Robert P. Smith, Pacific Islands Manager, U.S. Fish and Wildlife Service, Honolulu, Hawaii

Subject: Section 7 Consultation on Experimental Use of Diphacinone Pellets in Hawaii Volcanoes National Park.

Ref: Biological Opinion (Log Number 1-2-99-F-03)

This represents the biological opinion of the U.S. Fish and Wildlife Service (Service) in accordance with section 7 of the Endangered Species Act of 1973 (16 U.S.C. 1531-1544; Stat. 884) as amended, (Act) regarding potential impacts to the endangered Hawaiian hawk or Io (*Buteo solitarius*) from use of diphacinone pellets in Hawaii Volcanoes National Park (HAVO). Hawaii Volcanoes National Park is proposing to test hand-broadcasting of diphacinone pellets (Ramik Green), a multiple-feeding, anticoagulant rodenticide, for control of rats in wet forests of the Park. The test would take place in a 10-acre experimental plot in Kipuka Ki and another 10-acre plot in Olaa Forest. This research could lead to registration of diphacinone pellets for rat control in conservation areas of Hawaii. Currently, the anticoagulant diphacinone in pellet form is registered for use in residential areas in Hawaii, but not for use in conservation areas. The research will be conducted by Gerald Lindsey and David Foote, both of the U.S. Geological Survey (USGS), Biological Resources Division (BRD).

This biological opinion is based upon: 1) the Biological Evaluation Form and letter requesting consultation, received by us on May 19, 1999, dated May 17, 1999; 2) information provided in the Service's Hawaiian Hawk Recovery Plan; 3) other biological literature (see References at the end of the document); and, 4) information contained in our files. Our log number for this consultation is 1-2-99-F-03. Copies of pertinent materials and documentation are maintained in an administrative record in the Service's office in Honolulu, Hawaii.

CONSULTATION HISTORY

There has been no prior consultation for this specific project. However, the following two recent, related projects did require formal consultation due to the possibility of their effect on Io:

(1) Biological Opinion (Log Number 1-2-97-F-10)
HAVO requested Section 7 consultation to conduct testing of brodifacoum, a single-feeding, anticoagulant rodenticide, for control of rats in two 4-ha study areas within the Park's Koa Unit of Olaa, Island of Hawaii. It was the opinion of the Service that the proposed study was not likely to jeopardize the continued existence of the Io.

(2) Biological Opinion (Log Number 1-2-94-F-04)
Kamehameha Schools Bishop Estate (KSBE) and the National Biological Survey requested a Service opinion for a proposed study to evaluate the mortality and fate of rats poisoned with Eaton's All-Weather Bait Blocks rodenticide in the KSBE's Keahou Ranch and Kilauea Forest lands on the Island of Hawaii. It was the opinion of the Service that the proposed study was not likely to jeopardize the continued existence of the Hawaiian hawk.

BIOLOGICAL OPINION

Description of the Proposed Action

Rats are considered one of the primary factors responsible for the decline of Hawaiian endemic forest birds and snails. Rats and mice are also thought to have limiting impacts on some species of rare plants and invertebrates in Hawaii. The Service is, therefore, strongly supportive of efforts to control rodents in forested areas of Hawaii. The study proposes to determine the mortality and fate of rats following placement of diphacinone pellets, and will be very useful in evaluating future registrations of this product as well as in developing proper protocols for further use of rodenticides in Hawaii's forested areas in order to minimize the potential for secondary poisoning of non-target species.

BRD proposes to test the efficacy of pelletized diphacinone baits to control rats. A rat toxicant Ramik green bait (0.005% diphacinone), will be applied to a 10-acre plot in Kipuka Ki and a 10-acre plot in Olaa forest. Untreated control plots will be established nearby in Olaa forest and Kipuka Puaulu. The 3/4 inch long pellets will be hand-broadcast up to four times per year during the first year of the two year study. Bait application may continue in a second year focusing on non-target effects. BRD researcher Gerald Lindsey will test the toxicant's efficacy and the fate of poisoned rats. BRD researchers David Foote and Linda Pratt will evaluate effects on invertebrates and plants.

Biology and Population Status of the Species

The action area is occupied by the endangered Io, Hawaiian hoary bat (*Lasiurus cinereus semotus*), and Ou (*Psittirostra psittacea*), as well as three endangered plant taxa, the Oha wai (*Clermontia peleana*), the Anunu (*Sicyos alba*), and the Ha iwale (*Cyrtandra giffardii*). Plant species of concern also present in the area include, the Aku (*Cyanea tritomantha*), *Phyllostegia foribunda*, *Stenogyne macrantha*, *Stenogyne schrophulariodes*, and *Schiedea diffusa*. The only federally listed species in the action area that may be adversely affected by diphacinone is the Io.

Unless otherwise referenced, the following information on the status and habitat requirements of the Io is taken from the Service's Recovery Plan for the Hawaiian Hawk dated May 9, 1984, the Hawaiian hawk population survey report dated July 2, 1994, prepared by the Western Foundation of Vertebrate Zoology, and the Demographic Studies and Population Surveys of the Hawaiian Hawk 1998 Annual Report written by Klavitter and Marzluff.

The Hawaiian hawk is endemic to the island of Hawaii. It was listed as endangered in the mid-1960's because of its apparently low abundance island-wide. However, in the mid-1980's the population appeared to be stable and was estimated to number between 1400 and 2500 birds (Griffin 1985). A more recent study by Morrison *et al.* (1994) estimated a density of 0.004 hawks per hectare across the island and a total of 1600 birds (1120 adults; 560 pairs). The most recent study estimates a population of 1,233 individual adult and young adult birds based on detailed spot maps and extrapolated to entire known range (Klavitter & Marzluff 1998). Io are known to feed on black rats (*Rattus rattus*), Polynesian rats (*Rattus exulans*), and Norway rats (*Rattus norvegicus*), as well as the house mouse (*Mus musculus*) and native and exotic birds.

Environmental Baseline

The environmental baseline describes the status of the species and factors affecting the environment of the species or critical habitat in the proposed action area contemporaneous with the consultation in process. The baseline includes State, local, and private actions that affect a species at the time the consultation begins. Unrelated Federal actions that have already undergone formal or informal consultation are also a part of the environmental baseline. Federal actions within the action area that may benefit listed species or critical habitat are also included in the environmental baseline.

Recent Past and Ongoing Studies in HVNP Which May Affect/Have Affected Io:

1. From approximately 1980-1983, Kurt Griffin conducted reproductive biology and ecology studies of Io, which involved close nest monitoring, nest tree surveys, and tracking of adults by radiotelemetry in HAVO.

2. Darcy Hu of HAVO began an ongoing study in October 1995 testing the effects of diphacinone rodenticide (in the form of Eaton's All-Weather Blocks) on rat and mongoose control in brooding and nesting areas of nene. The tests take place in several areas of HAVO including Ainahou, Kipuka Nene, Kilauea summit area, and coastline at the end of Chain of Craters Road. Negative effects on Io have not been observed.

3. In June of 1997, a pair of Io were translocated by Service biologists from the Kona side of the island to the HAVO for a behavior observation study. The pair eventually returned to the Kona area within a period of one month.

4. From September 1998 through December 1999, Gerald Lindsey of BRD conducted a study testing the effects of brodifacoum, a single-feeding, anticoagulant rodenticide, for rat control in the Olaa Forest of the HAVO.

5. From approximately November 1998 through February 1999, Darcy Hu of HAVO conducted an East Rift (within HAVO) fence construction survey using auditory playback of Io calls to ensure that actual fence construction would not disrupt any active nests on/near the proposed fenceline.

6. John Klavitter with the University of Washington has been studying Io within the HAVO since the summer of 1998. His research involves monitoring mating pairs of Io, nest monitoring, and tracking individuals with transmitters. His research is not believed to

negatively impact the Io in any way. Klavitter recently estimated that approximately 1 to 2 adults may be found within the Olaa Forest, and 2 to 3 adults may be found in Kipuka Ki at any given time (personal communication 1999), which represents only about 13% of the known population within either of those areas.

Effects of Action on Listed Species

Io may be exposed to diphacinone by ingesting dying or dead rats or mice, which have eaten diphacinone. Laboratory tests indicate potential secondary poisoning hazards to raptors from poisoned prey species. Although no tests have been conducted on Io directly, tests have been conducted on North American raptors to determine efficacy of secondary poisoning by diphacinone. A 1980 study reported great-horned owls (*Bubo virginianus*) and saw-whet owls (*Aegolius acadicus*) succumbed when fed mice that died after feeding on 0.005% diphacinone-laced grain bait (Mendenhall *et al.* 1980). In another study, it was concluded that secondary poisoning of non-target species in Hawaii was unlikely. In this case, researchers found that residues of diphacinone in rats fed 0.00025% bait as their sole food source until death averaged 0.33 ppm in tissue samples, far less than the diphacinone concentration fed to raptors in other tests that did not cause toxicosis. It was suggested that under field conditions the availability of foods would reduce the possibility of consumption of contaminated prey (Keith *et al.* 1990).

In a 1994 study, Lindsey and Mosher assessed the secondary hazard potential of diphacinone to raptors, particularly the endangered Io and the short-eared owl or Pueo (*Asio flammeus sandwichensis*), within forested areas. Their study results suggest hazards to avian predators from baiting with 0.005% diphacinone bait will be minimal. Furthermore, the non-target secondary poisoning hazard resulting from the use of diphacinone is reduced by the delay in death of the target species, which allows time for the cleaning of the gut content, metabolism and excretion of the toxicant (Godfrey 1995).

In their 1992 study, Cox and Smith showed that food and water intake declined rapidly in anticoagulant-treated, caged *Rattus norvegicus*. In a different study, Hooker and Innes (1995) reported that black rats poisoned with the anticoagulant brodifacoum maintained normal nocturnal movements and showed no nest change between dawn and dark, suggesting no daytime movements. Apparently, most rats died in their nests or under cover, further suggesting that few rats dying of anticoagulant poisoning would be found in the open (Hooker and Innes 1995, Lindsey and Mosher 1994).

On the other hand, another study showed that between 20% and 50% of radio-collared rats poisoned by diphacinone and chlorophacinone in Florida sugarcane fields died in "exposed" areas and were available for scavenging by mammalian predators. It was also found that poisoned rats became comatose 1-2 days before death, leaving them more vulnerable to predation. There was no assessment to quantify the risk to raptors (Labisky *et al.* 1986). Additionally, in a 1992 study, Cox and Smith observed that diurnal activities increased almost 30% in caged rats treated with anticoagulants. Within a 24 hour period before death, these rats spent much of their time staggering or sitting quietly in the open, potentially increasing their vulnerability to raptor predation.

Although very little research has focused on the effects of diphacinone on arthropods, the few past studies suggest that arthropods themselves are not greatly affected, but may be capable of secondarily poisoning small birds or mammals when fed upon (Extoxnet 1993; Anonymous 1996;

Godfrey 1985). Furthermore, in a recent preliminary study on the effects of brodifacoum on large-headed wetas (*Deinacrida* spp.), results suggest that insects and other arthropods are capable of rapidly metabolizing and excreting the toxicant (Morgan and Wright 1995). Results from these studies suggest that: 1) dead rodents are located rapidly by mammalian scavengers; 2) raptors do not appear to recognize dead rodents lying on the forest floor as food items; 3) some rats may move above ground during the day, before and after consuming diphacinone bait, but generally remain under cover, minimizing their exposure to avian predators; 4) diphacinone contaminated rats are available for scavenging for only a short duration, and 5) due to the availability of a wide range of prey, and the small treatment area, the potential risk for injury or death to Io as a result of poisoning from diphacinone, is extremely low to nonexistent.

Cumulative Effects

Cumulative effects include the effects of future State, local, or private actions that are reasonably certain to occur in the area considered in this biological opinion. Future Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act. The Service has not identified any cumulative effects in the project area that may impact the Io.

Conclusion

After reviewing the current status of the Io (*Buteo solitarius*), the effects of the proposed study, and the cumulative effects, it is the Service's biological opinion that the study, as proposed, is not likely to jeopardize the continued existence of the Io (*Buteo solitarius*). No critical habitat has been designated for this species, therefore none will be affected. Additionally, the rodent control program may be beneficial for a number of endangered species in the project area.

INCIDENTAL TAKE

Section 9 of the Act and Federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered or threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavior patterns which include, but are not limited to, breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, carrying out an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered a prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

The measures described below are non-discretionary, and must be undertaken by the National Park Service for the exemption in section 7(o)(2) to apply. The National Park Service has a continuing duty to regulate the activity covered by this incidental take statement. If the National Park Service fails to assume and implement the terms and conditions, the protective coverage of section 7(o)(2) may lapse. In order to monitor the impact of incidental take, the National Park Service must report

the progress of the action and its impact on the species to the Service as specified in the incidental take statement. [50 CFR S402.14(i)(3)]

Sections 7(b)(4) and 7(0)(2) of the Act do not apply to the incidental destruction of listed plant species. However, protection of listed plants is provided to the extent that the Act requires a Federal permit for removal or reduction to possession of endangered plants from areas under Federal jurisdiction, or for any act that would remove, cut, dig up, or damage or destroy any such species on any other area in knowing violation of any regulation of any State or in the course of any violation of a State criminal trespass law.

Amount or Extent of Take

The Service anticipates that one (1) Io could be taken as a result of this proposed action. The incidental take is expected to be in the form of death from secondary poisoning.

Effect of the Take

In the accompanying biological opinion, the Service determined this level of anticipated take is not likely to jeopardize the continued existence of the Io. The study is being undertaken in a limited area so that even if one Io is secondarily poisoned, the island-wide population is sufficiently viable to tolerate such a loss.

Reasonable and Prudent Measures

The Service believes the following reasonable and prudent measures are necessary and appropriate to minimize impacts of incidental take of the Io. The National Park Service must ensure that BRD monitors Io and the fate of poisoned rats and mice in the study area and minimizes the likelihood of secondary poisoning.

Terms and Conditions

In order to be exempt from the prohibitions of section 9 of the Act, the National Park Service must comply with the following terms and conditions, which implement the reasonable and prudent measure described above. These terms and conditions are non-discretionary.

- Keep trained observers in the field to take field notes and make follow-up observations on Io feeding in treated areas to document any evidence of mortalities, or signs of illness, such as external bleeding, unresponsiveness to humans, or other unnatural behavior.

- Io exhibiting signs of illness should be captured and treated with vitamin K (an effective antidote) and taken to a qualified avian veterinarian.

- Follow poisoned rats and mice to determine whether these animals are available to Io as easy prey. Remove poisoned rats and mice from the study area once they have been located.

- Document all of these observations and provide the Service with information regarding potentially adverse effects.

- Notify the Service (808/541-3441) within 3 working days if any take of Io occurs.

- Dead Io shall be properly salvaged and sent to Dr. Thierry Work of the National Wildlife Health Research Center (808/541-3445) for necropsy and analysis. Any specimens available following necropsy and scientific analysis shall be deposited with the B.P. Bishop Museum, 1525 Bernice St., Honolulu, HI 96817 (telephone: 808/547-3511). If the B.P. Bishop Museum does not wish to accession the specimens, the National Park Service should contact the Service's Division of Law Enforcement in Honolulu, Hawaii (808/541-2681) for instructions on disposition.

The Service believes that no more than one (1) Io will be incidentally taken as a result of the proposed action. The reasonable and prudent measures, with their implementing terms and conditions, are designed to minimize the impact of incidental take that might otherwise result from the proposed action. If, during the course of the action, this level of incidental take is exceeded, such incidental take represents new information requiring reinitiation of consultation and review of the reasonable and prudent measures provided. The Federal agency must immediately provide an explanation of the causes of the taking and review with the Service the need for possible modification of the reasonable and prudent measures.

Conservation Recommendations

Section 7(a) (1) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information. At this time, the Service has no recommendations.

Reinitiation-Closing Statement

This concludes formal section 7 consultation on this action. As required in 50 CFR 402.16, reinitiation of consultation is required where discretionary Federal agency involvement or control over the action has been retained (or is authorized by law) and if: 1) the amount or extent of incidental take is exceeded; 2) new information reveals effects of the agency action that may affect listed species in a manner or to an extent not considered in this opinion; 3) the agency action is subsequently modified in a manner that causes an adverse affect to the listed species that was not considered in this opinion; or 4) a new species is listed or critical habitat designated that may be affected by this action. In instances where the amount or extent of incidental take is exceeded, any operations causing such take must cease pending reinitiation.

If you have any questions regarding any of the information contained in this biological opinion, please contact either Assistant Field Supervisor Karen Rosa or biologist Mike Richardson (phone: 808/541-3441; fax: 808/541-3470).

cc: Larry Salata, RO-ES, Portland, OR

Robert P. Smith

REFERENCES

Anonymous. 1996. Application of anticoagulant toxic baits for rat eradication: background information and assessment of effects. Unpublished Report, Department of Conservation, Wellington, NZ.

Cox, P. and R.H. Smith. 1992. Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behavior. Proc. 15th Vertebrate pest Conf. J.E. Borrecco and R.E Marsh, eds. University of Calif. - Davis

Extoxnet. 1993. Diphacinone. Extoxnet: Extension Toxicology Network. Pesticide Management Education Program, Cornell Univ., NY.

Godfrey, M.E.R. 1995. Non-target and secondary poisoning hazards of "second generation" anticoagulants. Acta Zool. Fennica 173: 209-212.

Griffin, C.R. 1985. Biology of the Hawaiian Hawk *Buteo solitarius*. PhD. Dissertation, University of Missouri, Columbia.

Hooker S. and J. Innes. 1995. Ranging behaviour of forest-dwelling ship rats, *Rattus rattus*, and effects of poisoning with brodifacoum. New Zealand J. Zool. 22:291-304.

Keith, J.O., D.N. Hirata, D.L. Espy, S. Greiner, and D. Griffin. 1990. Field evaluation of 0.00025% diphacinone bait for mongoose control in Hawaii. Denver Wildlife Research Center, Denver, CO. 52 pgs.

Klavitter, J. and Marzluff, J. 1998. Demographic Studies and Population Surveys of the Hawaiian Hawk Annual Report. Submitted to the U.S. Fish and Wildlife Service, Pacific Island Office, Honolulu. 26 pgs.

Labisky, R.F., N.R. Holler, and R.J. Newberry. 1986. Potential hazards of diphacinone and chlorophacinone rodenticides to selected vertebrates in Florida sugarcane. USDA Report, Southern Region Pesticide Impact Statement Program, University of Arkansas.

Lindsey, G.D. and S.M. Mosher. 1994. Tests indicate minimal hazard to Io from diphacinone baiting. Hawaii's Forest and Wildlife 9(4):1, 3.

Mendenhall, V.M. and L.F. Pank. 1980. Secondary poisoning of owls by anticoagulant rodenticides. Wildl. Soc. Bull. 8:311-315.

Morgan, D.R. and G.R. Wright. 1995. Environmental effects of rodent Talon baiting. Part 1: monitoring for toxic residues. Landcare Research Contract Report: LC9495/142, Manaaki Whenua - landcare Research, New Zealand.

Morrison, M., L.S. Hall, and P.H. Bloom. 1994. Final Report Hawaiian Hawk (*Buteo solitarius*) Population Survey. Submitted to the U.S. Fish and Wildlife Service, Pacific Island Office, Honolulu.

U.S. Fish and Wildlife Service. Hawaiian Hawk Recovery Plan. Prepared by C.R. Griffin. May 9, 1984. 48 pgs.

Appendix 10. Environmental assessment report.

ENVIRONMENTAL ASSESSMENT: TEST BROADCAST RODENTICIDE RAMIK GREEN TO CONTROL RATS, HAWAI`I VOLCANOES NATIONAL PARK, HAWAI`I

September 3, 1999

Prepared by: _____ Date: 9/3/99

Approved by: _____ Date: 9/3/99

PURPOSE AND NEED

Safe, effective, and efficient methods of rat control are needed in Hawai`i Volcanoes National Park (HAVO) and other conservation areas in Hawai`i. This need has been identified as a high priority by the Secretariat for Conservation Biology and by state and federal conservation agencies. All rat species in Hawai`i are introduced and have harmful impacts on native birds, invertebrates, and plants. Recovery of many native species is expected with rat control. Current control methods available for use in conservation areas include snap trapping and a rodenticide deployed in bait stations. These methods are very labor intensive and practical only in very small, accessible sites. Broadcast rodenticide baits, namely those that are spread outside of a designated bait dispenser, are used in Hawai`i in agricultural settings and for rodent control around and in dwellings. Aerially broadcast rodenticides have also been used successfully in conservation areas in New Zealand. Research is required for regulatory agencies to modify pesticide labels to allow use of a broadcast rodenticide in conservation areas in Hawai`i; Research is needed to demonstrate the effectiveness of the rodenticide in controlling rats and not harming other species.

Three rats species are present in Hawai`i and HAVO. Black rats (also called roof rats or ship rats) (*Rattus rattus*) are the most widespread and abundant rat species in the park, particularly in mesic (moderately wet) forest and rain forest. Polynesian rats (*Rattus exulans*), which accompanied early Polynesian settlers throughout the Pacific, are generally less abundant than black rats and reach their highest densities in the lowlands. Norway rats (*Rattus norvegicus*) are found in many park environments but are never common.

Studies and observations of biologists suggest that rats have had powerful, negative effects on a number of Hawaiian native species. The impacts of black rats are probably of the greatest interest to resource managers. Black rats are arboreal and therefore can consume maturing fruits and prey on nesting birds. In addition they are abundant in montane mesic and wet forests, which are of the greatest conservation interest to park managers because of their intactness, manageability, and biological diversity.

The impact of rats on invertebrates and plants is suggested by the broad range and high volume of invertebrates, fruits, and seeds they consume (Sugihara 1996; Forbes and Stone *et al.*, draft manuscript). Park biologists have documented a wide range of fruits eaten by rats (Stone 1985, Russell 1980) and girdling of rare trees (Baker and Allen, 1978). Rats are considered a factor in the decline of bird faunas of many Pacific islands including Hawai`i (Atkinson 1977, Scott *et al.* 1986). In HAVO, the native Hawaiian bird `Elepaio (*Chasiempis sandwichensis*) must re-nest up to eight times because of egg predation by black rats (Sarr *et al.*, 1998). Rats are predatory on native Hawaiian tree snails and appear to be a major limiting

factor for these Endangered invertebrate species (Miller and Hadfield, 1993).

Few long-term studies of recovery after rat removal have been attempted. However, the short-term studies conducted suggest that native species may recover if rats are removed. Nesting success of ʻElepaio increased in an experimental control program on Oʻahu where rats were controlled (VanderWerf, 1997). Studies of recovery of rare forest bird populations are underway at Hakalau Forest National Wildlife Refuge (HFNWR), but no conclusions have been drawn from this study to date. A study after rat control on a small, off-shore island in New Zealand, indicate that seedlings and saplings of many native tree and shrub species increased substantially after five years of rat control (Allen, et al., 1994).

Broadcast rodenticides, widely used in agricultural settings and in and around dwellings, have potential for becoming an important management tool in conservation areas. There are over 40 rodenticides labeled for use in Hawaii (Swift 1997). Research is needed before resource managers can use broadcast rodenticides in conservation areas. Studies will indicate if broadcast rodenticides are effective, safe for non-target organisms, and stimulate the recovery of native species. Effectiveness and safety for non-target species need to be demonstrated for the US Environmental Protection Agency (EPA) to allow registration of a pesticide for use in conservation areas.

ALTERNATIVES INCLUDING THE PROPOSED ACTION AND THE AFFECTED ENVIRONMENT

Alternative 1. Proposed Action.

The next step in rat control for conservation agencies is to seek registration from EPA for the use of broadcast rodenticides. Broadcast rodenticides, especially those distributed by helicopter, have the potential to be more cost-effective over larger areas. When larger areas are treated, the reinvasion of rats from outside areas is reduced. The diphacinone broadcast bait Ramik Green (HACO, Inc, Madison, WI) is currently approved for use in and around dwellings and agricultural lands in Hawaiʻi. It is approved as pellets out of the reach of children and wildlife or in tamper-proof bait stations. In these settings, Ramik Green is effective with diphacinone as its active ingredient at 0.005%. Conservation agencies want to evaluate the efficacy and safety of Ramik Green as a broadcast bait for rat control in native forests. The target areas are localized upland breeding bird habitats and other biological communities where rare plants or affected species are concentrated. Broadcast applications of rodenticide over thousands of acres of continuous habitat are not envisioned.

Conduct research in Kīpuka KI and `Ōla`a Forest in Hawai`i Volcanoes National Park using broadcast baits. This research will start with a single 10-acre plot in each site under an Experimental Use Permit issued by the Hawai`i Department of Agriculture. Hand broadcasting of Ramik Green rodenticide will be carried out. Treatment sites will be paired with untreated controls in KĪuka Puaulu and a nearby area in `Ōla`a forest. This research is planned for fall, 1999. If the research proceeds successfully in the 10-acre test plots, permission will be sought from EPA to test one 50-acre plot each in `Ōla`a and Kīpuka KI. In these larger plots, baits will be dispersed by helicopter. This research is planned for fall, 2000. Research results about control efficacy and non-target effects will be described in reports and scientific papers. Another EA would be prepared before management implementation over larger areas, based on these reports and papers.

The research will be conducted by the Biological Resources Division of the U.S. Geological Survey. Principal investigators will be Dr. Gerald Lindsey and Dr. David Foote. Plots treated with the rodenticide will be in moist forest of Kipuka Ki and in rain forest of `Ola`a.. Untreated control plots will be located in Kīpuka Puaulu and a nearby area of `Ōla`a. Research methodology in the 10-acre test plots includes hand-broadcasting during dry weather at 10 lbs/acre to 30 lbs per acre, based on results of a field trial being conducted outside the park to determine the optimum bait application rate. Timing of repeat applications will depend on disappearance of bait and recovery or reinvasion of the rat population. For test purposes, baits will be replenished every 2-3 months regardless or when rats recover to pretreatment levels, whichever is longer. A maximum of four broadcast baitings will be applied to each treatment plot.

The objective is to reduce rat populations 60-90%. The 10-acre size for the treatment site imposed by the State Department of Agriculture is too small for rat control research because of rapid re-invasion. However, in order to qualify for research in a 50-acre site, probably the minimal area feasible for rat control, some success must be demonstrated in 10-acre plots.

Efficacy of treatments will be determined by trap-retrap, radio telemetry, and acceptance of nontoxic census blocks pre and post treatment. Live trapping of rats to get an estimate of the population size and species composition within the study areas will be conducted prior to baiting. Rodents captured will be ear-tagged and released. Snap trapping of mice will be conducted throughout the study to follow their population to determine if rat removal affects them. Ten to 15 rats in each 10-acre area will be fitted with radio transmitters to determine their fate. Dead radio-marked rats will be located, placed in plastic bags, and removed from the study area. Census blocks indicate the presence of rats by signs of chewing on the block.

Invertebrates. Pitfall traps and non-toxic pellets will be used to monitor effects of baiting on soil invertebrates. Impacts on invertebrates will be determined by a preliminary test using a non-toxic pellet to determine the attractiveness of baits to alien slugs, alien and native snails, and other invertebrates in the study areas. Individually marked pellets will be monitored daily for invertebrates. Pitfall traps are plastic containers placed in holes dug approximately 10 cm deep and 20 cm in diameter. Other invertebrates will be sampled throughout the study at coconut baits and sticky census boards. Some invertebrate specimens, including native and alien snails and slugs, the most likely species to be attracted to the baits, will be collected for residue analysis in order to evaluate potential for secondary toxicity.

Plants. The fate of seedlings, fruits, and buds of the following plant species will be tested: pilo (*Coprosma* spp.), hau kuahiwi (*Hibiscadelphus giffardianus*), *Clermontia hawaiiensis*, *C. parviflora*, kolea (*Myrsine lessertiana*), ho`awa (*Pittosporum hosmeri*), alani (*Melicope* spp.), papala kepau (*Pisonia brunoniana*), `ōlapa (*Cheirodendron trigynum*) and mamaki (*Pipturus albidus*). Four hundred seeds of these species will be germinated in the park greenhouse prior to the baiting experiment and outplanted to assess impacts of rats and rat control on seedlings. Some seedlings will be placed in rat-proof exclosures, an equal number will be protected from slugs, and some will be placed in exclosures that block access of kalij pheasants but not rats. Up to 50 flowers, fruits, and buds of the above species will be marked and monitored weekly in the control and treatment area, before and after baiting. Twenty seed traps, protected from seed predators, will be placed in each treatment area to help determine seed rain and seed predation under target tree species thought to be vulnerable to rat predation. Grain seeds have been observed to germinate from grain baits in the past. The potential of introducing weeds into the treatment sites will be evaluated by following germination and fate of seeds in grain baits in the greenhouse and under field conditions.

Birds. Up to 20 individuals each of the following alien bird species will be mist netted, euthanized, and then sent to a lab, and checked for pesticide residues. They will be euthanized using standard carbon dioxide methods or cervical dislocation: Northern Cardinal (*Cardinalis cadinalis*), Japanese White-eye (*Zosterops japonicus*) and Melodius Laughing Thrush (*Garrulax canorus*) will be removed using mist netting techniques (Master banding permit from USFWS #22613 issued to BRD). Up to 20 Kalij Pheasants (*Lophura leucomelana*) will be collected with the use of a shotgun in consultation with HAVO Protection Rangers. Transects will be walked inside and outside the study area to observe dead or poisoned birds. These will be necropsied and analyzed. Radio collared and banded `Ī`o in the area will be monitored.

This is the park's preferred alternative.

Alternative 2. Expand use of existing approved pesticides in bait stations. A rodenticide working group was formed in Hawai`i in 1994 to seek registration of rodenticides in conservation areas of the state. This interagency group was comprised of representatives from the Hawai`i Division of Forestry and Wildlife, Hawaii Department of Agriculture, US Geological Survey- Biological Resources Division, US Fish and Wildlife Service, US Department of Agriculture, Kamehameha Schools/Bishop Estate, and the National Park Service. In 1995 the efforts of the working group resulted in the registration of Eaton's All-Weather Bait Blocks for use in covered bait stations in conservation areas. The bait stations restricted access to the bait for birds, humans, and other large non-target species. The active ingredient of this rodenticide is diphacinone (0.005%), the same active ingredient that is in the Ramik Green pellets proposed for broadcast application. The working group targeted diphacinone as a likely candidate rodenticide because of its effectiveness against rats and low incidence of toxicity to non-target organisms. Diphacinone is an anticoagulant, the most commonly used type of rodenticide because of slow action, high palatability, low risk, and easy application (Lund 1988). Anticoagulants work by interfering with an organism's ability to utilize vitamin K_1 in the process of blood clotting and by damaging smaller blood vessels, causing internal bleeding.

Use of rodenticide in bait stations can be effective in only small, accessible areas. Bait stations are impractical for large or remote areas. Dipahcinone bait stations are not used systematically in any areas of HAVO because they are highly labor intensive to set out and maintain. They are currently used around several individuals of one species of endangered plant to prevent bark girdling during dry periods. They are also used around selected nēnē nesting and brooding areas to control mongoose and rats (mongoose are also highly sensitive to diphacinone).

Alternative 3. Expand use of snap traps. Use of baited snap traps can also be an effective method of rat control. However, they are even more labor intensive to maintain than are bait stations, making their use over large areas and in remote locations not feasible. Snap traps are currently not in use for rat control in HAVO.

Alternative 4. No action. Do not control rats.

Affected Environment.

Kipuka KI. Kīpuka KI is a mesic forest located on the southeast slope of Mauna Loa at approximately 4,200 foot elevation. It is bisected by the Mauna Loa Road. Rainfall is approximately 60 inches per year and the substrate is deep ash accumulated over the last 10,000 years. The kīpuka is divided by and surrounded by much younger lava flows from Mauna Loa. Kīpuka KI is dominated by stands of the native tree species koa (*Acacia koa*), mānele (*Sapindus saponaria*), and `ohi`a (*Metrosideros polymorpha*). Stands of mānele

are very rare in Hawai`i and all occur on the slopes of Mauna Loa. Rare native flies (*Drosophila* spp.) are associated with the mānele and koa. In scattered locations a subcanopy of native trees is present. The understory vegetation is largely alien, comprised of stands of alien grasses, blackberry, and Jerusalem cherry. In some areas, there are dense stands of native palapalai fern. An active habitat restoration program is underway using herbicides to suppress alien understory vegetation and stimulate the establishment of native plants. Kīpuka KI was subjected to cattle grazing and feral goats until the 1970s, and feral pigs were present until the 1980s. No endangered or threatened or species of concern are currently found in Kīpuka KI. The common native birds found on the Big Island are present including `Ōma`o (*Myadestes obscurus*), `Apapane (*Himatione sanguinea*), `Elepaio, and seasonally `I`iwi (*Vestiaria coccinea*). The endangered `Io or Hawaiian hawk (*Buteo solitarius*) is found in the lower Mauna Loa area, and nests are known from nearby Kīpuka Pua'ulu. The area is visited by Native Hawaiians harvesting traditional plant materials under a park permit system. Most of this is done adjacent to the road with the heaviest collecting done in April. No hunting is allowed.

`Ōla`a Forest. The `Ōla`a site is located adjacent and east of Wright Road above Volcano Village. The rain fall is approximately 140 inches per year. `Ōla`a forest is tree fern dominated rain forest. `Ōhi`a, the emergent tree species, occurs in open to scattered stands. Tree ferns form closed stands 10-15 feet tall, along with several other native tree species. The understory is comprised largely of native ferns and shrubs. `Io is occasionally seen in `Ōla`a forest. Common native birds are also present; no rare birds use the area. Two endangered plant species, `anunu (*Sicyos alba*) and ha`iwale (*Cyrtandra giffardii*) may occur in the area. Historically, in the early 1980's, a proposed endangered endemic picturewing fly (*Drosophila heteroneura*) occurred in the study area. Feral pigs were removed in the early 1990s, and no hunting is taking place. Alien plant control began in 1999 using herbicides. There are no established trails and hiking and other public uses are very uncommon.

ENVIRONMENTAL IMPACTS

Alternative 1. Test broadcast rodenticides.

Broadcast diphacinone is expected to result in 60-90% control of rats in test and management areas. Rats are very sensitive to diphacinone and have a lethal dose of 0.6 milligrams of diphacinone/kilogram of body weight. Under field conditions, rats must consume a sublethal dose for six straight days to die.

Human Health and Safety. The use of diphacinone in pellets at 0.005% is not expected to pose a threat to human health and safety. Diphacinone was first developed in 1957 as pharmaceutical called Dipaxin, an anticoagulant used to treat circulatory problems in

humans. It was developed as a rodenticide in 1972 because of its anticoagulant properties. An adult human would have to eat approximately 0.882 pounds of the bait or 34 pellets for an initial therapeutic dose and 3-7 pellets a day to maintain the therapeutic dose. The lethal dose for humans is not known because it has not been directly tested on humans to determine this. Dosages from accidental poisonings could not be found.

Domestic Dogs. There is little to no danger of secondary poisoning to pig hunters or their hunting dogs. The park will test and use broadcast baiting only in areas where pigs have been controlled and hunting does not occur. HAVO was selected as a study area because of its extensive pig-free areas. Pigs eating diphacinone baits do not accumulate this pesticide in muscle tissue. No residues of diphacinone were found in the muscle tissue of pigs fed diphacinone for 2-5 days at levels representing 120 to 300 pellets per feeding. Diphacinone was found in the liver but at such low levels that 10 tons of liver would need to be consumed to attain just the human therapeutic dosage. The study sites were deliberately selected to avoid the potential of conflicts with hunters. Hunters may potentially traverse the `Ōla`a study site with their dogs in accessing areas with pigs east of the fenced unit in which the treatment plot lies. Hunters almost invariably avoid the area surrounding the treatment plot because quicker access is located in pastures just outside the park. It is possible that stray or lost hunting dogs may wander through the treatment plots. Sometimes these dogs may spend one or more days in an area. The lethal dose is known for dogs: it is 3.0-6.5 milligrams/kilogram of body weight. An 11.78 gram pellet contains 0.589 milligrams of diphacinone. For a 30 pound (13.61 kilogram) dog to obtain the lower lethal dose of 3 milligram/kilogram, it would have to eat 69.8 pellets (40.83 milligrams diphacinone). To ingest the upper level dose, 7.75 milligrams/kilogram, a 30 pound dog would have to eat 173.3 pellets (102.07 milligrams). If these dogs were starving, they may possibly ingest large numbers of pellets if palatable. Stray dogs were not observed to eat pellets in a recent study using placebo Ramik Green pellets along Stainback Highway outside the park.

Vegetation. Trees, shrubs and other plants are not expected to be harmed by the baits. Seedling establishment of plant species most likely will be enhanced as rat predation of seeds and seedlings declines. Bark girdling may be reduced on papala-kepau (*Pisonia brunoniana*) and possibly other species. The effects of rat predation on seeds and seedlings of hau kuahiwi (*Hibiscadelphus giffardianus*), an endangered plant species, will be tested. Testing will involve manipulative research in which seedlings and seeds are offered to rats in the field. Grain species have been observed to germinate from grain pellets. This has the potential of introducing weeds into the treatment areas. Germination of seeds will be tested under optimal conditions in the greenhouse. Germination and fate of seedlings will be tested under field conditions.

Birds. Native forest perching birds are not expected to be impacted because they largely feed on nectar or insects above the forest floor. Native birds occasionally feed on the ground where they could come into contact with contaminated invertebrates. Owls feed on mice and rats. Peuo (*Asio flammeus sandwichensis*), the native owl, is not present in ʻŌlaʻa and very rare in Kīpuka KI. It resides and forages in more open areas. Alien barn owls (*Tyto alba*) are present in the lower Mauna Loa strip forests. Alien birds feeding on the forest floor may take pellets or invertebrates with residues of pesticide. Kalij pheasant is the species spending most of its time feeding on the ground. Pesticide residues will be checked in a number of bird species. Alien species from a variety of feeding guilds will sampled. If residues are found, then native birds may be also tested. Any suspicious dead birds in and near the study area will be tested for pesticide residue.

The effects of rat control on house mice (*Mus musculus*) populations are unknown. These rodents are present from the shoreline to alpine areas but are much less abundant than black rats and Polynesian rats in both of the test sites. Mice are uncommon in the rain forest except for grassy openings. House mice are little affected by diphacinone. Mice largely depend on a food base made up of small seeds and may not directly compete with rats. They may increase in numbers if rats are controlled. This is an important research question and mice populations will be monitored closely during the study.

Mongoose populations are expected to decline because they are very sensitive to diphacinone and are known to be attracted to this bait in bait stations. Feral cats may be attracted to the baits. The lethal dose for cats has been experimentally determined. Cats are less sensitive than dogs but weigh less. Approximately 50 pellets would be required for the average weight feral cat. There may be some hazard of secondary poisoning by feeding on dead or poisoned rats. Large numbers of rats would need to be ingested to receive the equivalent of the above dosages.

Invertebrates. Insects and other invertebrates are not known to be sensitive to diphacinone and no direct effects are expected from application of the rodenticide. However, endemic picture-wing Drosophila will be tested under laboratory conditions for attraction to the bait and impacts of the active ingredient. The baits will provide a food source for detritivores, particularly alien slugs and sowbugs and populations of these invertebrates feeding on the baits may increase. Snails and slugs will be tested for pesticide residue.

Threatened and Endangered Species (Section 7 Consultation). Endangered ʻĪo are not expected to be harmed through secondary toxicity by scavenging or preying on rats which have ingesting diphacinone). ʻĪo are resident in the lower Mauna Loa Strip and are occasionally seen in ʻŌlaʻa. Studies at HFNWR (Lindsey and Mosher 1994) indicate that 1) dead rats are rapidly located by mammalian

scavengers such as rats, mongoose, and cats; 2) rats are largely nocturnal and those moving around in the day tend to remain under cover; 3) diphacinone contaminated rats tend to die in their nests, not in the open. A Section 7 consultation was carried out the US Fish and Wildlife Service (FWS). The FWS has provided a Biological Opinion, concluding that the proposed study is not likely to jeoparadize the continued existence of the ʻĪo and that the control proram will be beneficial to other endangered species. The FWS has prescribed terms and conditions in the Biological Opinion requiring close monitoring for signs of toxicity in ʻĪo, tracking rat movement, and necropsy of dead birds. An incidental take of one bird is allowed. Impacts on hau kuahiwi will be mitigated by the fact that surviving individuals will be left in the ground to augment the existing population in the nearby Kīpuka Puaulu.

Wilderness. Wilderness will be affected by diphacinone baiting. The Ola'a site is located in wilderness; the Kīpuka Kī site is not. Other wilderness sites could be targeted for baiting if registration of the pesticide is achieved. Impacts that could affect wilderness values include the use of pesticides, flagging tape, markers, and plastic pitfall traps, and helicopters to broadcast baits. These intrusions on wilderness are balanced by the potential gain of restoration of ecological integrity and native species recovery. Hawaii's natural areas, including designated wilderness, are imperiled by alien species. Hawai`i's wilderness/resource managers have perceived use of fences, pesticides, and occasionally helicopters as the necessary minimum tool to accomplish the objective of restoring wilderness ecological integrity, habitat restoration, and native species recovery and protection.

Cultural Resources. The research will be carried out to not impact cultural resources. Holes four inches deep and eight inches wide will be excavated. The siting of these holes will be reviewed with a park archeologist to avoid impacts to archeological resources, if any are present. Plants collected by Native Hawaiians in Kīpuka Ki, e.g., palapalai fern (*Microlepia strigosa*) will not be affected by diphacinone.

Soil and Water. Effects on soil and water are short-lived and not pervasive. Diphacinone is readily adsorbed by soil organic matter so it does not move in the soil. It is not readily soluble in water and therefore disperses little in water. It breaks down to harmless, simple compounds in both environments. It has a half-life (half of original amount breaks down) in soil of 35 days.

Alternative 2. Use bait stations.

The effects on black rats are similar to those of broadcast baiting. Bait delivery to Polynesian rats would need to be modified to achieve similar levels of rat control expected on black rats. Researchers at HFNWR were not successful in controlling Polynesian rats with commercial, box style, bait stations. If all species of rats can

effectively be controlled, similar effects on vegetation, invertebrates, birds, and other mammals can be expected. Helicopters use will be reduced or not needed in the 50 acre test plots and in follow up management use if broadcast rodenticides are not used. Helicopters may be needed to carry large volumes of bait stations initially needed into remote sites. For small areas, bait stations probably require similar levels of effort to put out and replenish as hand broadcast baits. Influx of rats from untreated areas is great in small areas requiring frequent replenishing of baits, whether hand broadcast or delivered in bait stations. However, for larger target sites, e.g., greater than 50 acres, broadcast baiting will be greatly more cost-effective because of the use of helicopters to deliver baits and because of the lower edge-to- area ratio and lower reinvasion rates. Using bait stations in more remote areas or areas with rugged terrain is much less cost-effective than using helicopters. Helicopter dispersal reduces the number of personnel needed and travel time to and within the site to deliver and replenish bait.

Human Health and Safety. The active ingredient (diphacinone 0.005%) for the rodenticide used in bait stations is identical to that used in Ramik Green pellets. As in Alternative 1., the use of diphacinone at 0.005% is not expected to pose a threat to human health and safety.

Domestic Dogs. Bait stations are designed to prevent large vertebrates from gaining access to baits. If a bait station were overturned, some bait could spill out. However, the opportunity for dogs to eat the bait is greatly reduced when compared with broadcast pellets.

Vegetation. Similar to Alternative 1., the use of diphacinone baits is not expected to have any impact on vegetation.

Wildlife. Only the house mouse is likely to be able to access the baits when placed in bait stations. The impacts on mice populations of controlling rats will still need to be addressed.

Invertebrates. Alien sow bugs, slugs and other invertebrates are attracted to rodenticide baits in stations, and the consequences of baiting will need to be evaluated as in Alternative 1.

Threatened and Endangered Species (Section 7 Consultation). Section 7 consultations will be sought as in Alternative 1.

Wilderness. The intrusions on wilderness are likely to be similar to those described under Alternative 1. for broadcast toxicants, except that bait stations will be employed instead of broadcast pellets.

Cultural Resources. No impacts on cultural resources are expected under this alternative.

Soil and Water. No impacts on soil or water are expected under this alternative.

Alternative 3. Use Snap traps.

Snap trapping is effective and similar recovery can be expected. However, it is extremely labor-intensive, especially in larger areas and remote areas. There is the potential to learn about recovery from these kinds of studies.

Human Health and Safety. Snap traps for rats can be a hazard if accidentally touched. Traps would need to be deployed with adequate warning signs so that traps can be avoided.

Domestic Dogs. Coconut and peanut butter are commonly used to bait snap traps for catching rats. If dogs were attracted to these baits and accidentally triggered the snap trap, they could be injured.

Vegetation. Snap trapping would have no impact on vegetation.

Wildlife. Snap traps will probably not harm other wildlife, but the hazards will need to be evaluated.

Invertebrates. Alien slugs and other invertebrates are attracted to baits on snap traps. The impact of deploying baits on invertebrate populations will need to be monitored.

Threatened and Endangered Species (Section 7 Consultation). Section 7 consultations will be sought as in Alternative 1.

Wilderness. The intrusions on wilderness are likely to be similar to those described under Alternative 1. for broadcast toxicants, except that snap traps will be employed instead of broadcast pellets.

Cultural Resources. No impacts on cultural resources are expected under this alternative.

Soil and Water. No impacts on soil or water are expected under this alternative.

Alternative 4. No action.

Do not control rats. This describes current management. No potential negative impacts of using rodenticide will occur. However, recovery of native species will continue to be adversely affected by rats.

MITIGATION

Managed areas will be marked with warning signs; known user groups will be informed. All flagging, markers, and pitfall traps will be removed at the end of the study. Archeologist will accompany field technicians in locating holes for pit fall traps. These holes will be filled when the study is complete. Broadcast baits will be used only in areas without game animals and hunting activity. Effects on non-target species will provide mitigation of impacts. Pesticide residues will be checked in a wide range of alien bird species representing different feeding guilds. Study area and vicinity will be systematically checked for sick and dead birds, which will be necropsied and analyzed. Bait attractiveness and impacts of pesticide on Drosophila will be monitored in laboratory studies. Grain in bait will be tested for germination of weed seeds. Pesticide residues in slugs and snails attracted to the bait will be tested.

Literature Cited

Allen, R.B., W.G. Lee, B.D. Rance. 1994. Regeneration in indigenous forest after eradication of Norway rats, Breaksea Island, New Zealand. New Zealand Journal of Botany, 32:529-439.

Atkinson, I.A.E. 1977. A reassessment of factors, particularly Rattus rattus, that influenced the decline of endemic forest birds in the Hawaiian Islands. Pacific Science 31:109-133.

Baker, J.K. and M.S. Allen. 1978. Roof rat depredations on Hibiscadelphus (Malvaceae) trees. Pp. 2-5 in C.W. Smith (ed).) Proc. 2nd Hawaii Volcanoes National Park Natural Sciences Conf.

Forbes, C.M. and C.P. Stone. In prep. Population characteristics, diets, and feasibility of localized short-term control by chemical means of introduced rodents at Hawaii Volcanoes National Park, Hawaii.

Lindsey, G. D. and S.M. Mosher. 1994. Tests indicate minimal hazard to `Io from diphacinone baiting. Hawai`i's Forests and Wildlife 9(4):1,3.\

Lund, M. 1988. Anticoagulant rodenticides. In I. Prakash (ed.), Rodent Pest Management. CRC Press, Inc. Pps. 341-351.

Miller, S.E. and M.G. Hadfield. 1993. Conservation of Hawaiian tree snails. Abstract In 1993 Hawaii Conservation Conference.

Russell, C.A. 1980. Food habits of the roof rat (*Rattus rattus*) in two areas of Hawaii Volcanoes National Park. P.p. 269-272 in C.W. Smith (ed) Proc. 3rd Conf. In Natural Sciences, Hawaii Volcanoes National Park.

Sarr, Z, N.P. Shema, and C.P. Stone. 1998. Nesting success and population status of the `Elepaio (*Chasiempis sandwichensis*) in the Mauna Loa Strip Section of Hawai`i Volcanoes National Park. Technical Report 118. Coop. Parks Stud. Unit, Univ. of Hawaii, Honolulu.

Scott, J.M., S. Mountainspring. F.L. Ramsey, and C.B. Kepler. 1986. Forest bird communities of the Hawaiian Islands; their dynamics, ecology, and conservation. Stud. Avian. Biol. 9.

Sugihara, R.T. 1997. Abundance and diets of rats in two native Hawaiian forests. Pacific Science 51:189-198.

Swift, C. 1996. A proposal to study the foraging ecology and microhabitat use of introduced rats in upper-elevation native Hawaiian forests. Report in park files.

VanderWerf, E. A. 1997. Demography of the Oahu `Elepaio as a guide to conservation. Abstract In 1997 Hawaii Conservation Conference.

PERSONS CONSULTED

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ENVIRONMENTAL IMPACTS MATRIX

	ALTERNATIVE 1	ALTERNATIVE 2	ALTERNATIVE 3	ALTERNATIVE 4
	BROADCAST BAITS	BAIT STATIONS	SNAP TRAPS	NO CONTROL
IMPACT TYPE				
Cultural Resources	4 X 8 inch holes dug	No holes dug	No holes dug	No holes dug
Water Resources	Pesticide insoluble in water so not carried in soil water	No contact with water	No pesticide used; no effects	No pesticide used; no effects
Soil	Half life of 35 days in soil	No contact with soil	No pesticide used; no effects	No pesticide used; no effects
Wilderness	Intrusion from use of pesticide, research activities, helicopters; potential improvement of ecological integrity in wilderness with native species recovery	Intrusion from use of pesticides, bait stations; potential improvement of ecological integrity in wilderness with recovery of native species.	Intrusion of using traps potential improvement of ecological integrity in wilderness with recovery of native species.	No impacts from research and use but also no potential benefit to ecological integrity of wilderness
Threatened and Endangered Species	Potential impact of rat chewing on outplanted *H. giffaradianus*; potential increase in *H. giffardianus* population; potential benefit to other E&T plants through rat control; potential secondary poisoning of 'Io	Potential in increased regeneration of E&T plant species; potential for secondary poisoning of 'Io	Potential for increased regeneration of E&T plant species; no potential for secondary poisoning of 'Io.	Continue rat impacts on endangered plant species; no potential for secondary poisoning of 'Io.
Invertebrates	No known direct effects but may be increase in population of slugs, snails feeding on bait	No direct effects but may be increase in population of slugs, snails feeding on bait	No effects	No effects
Birds	Little potential effects on native birds; potential intake of pesticide by ground dwelling alien species	No effects	Birds can get caught in snap traps	No effects
Plants	Potential for reduced bark girdling and seed predation; potential for germination of grain seeds in bait	Potential for reduced bark girdling and seed predation; potential for increased regeneration	Potential for reduced bark girdling and seed predation; potential for increased regeneration	No potential for improved regeneration
Human Health and Safety	Potential poisoning to small children who eat large number of pellets	Potential poisoning to small children if shake bait out of boxes and ingest	Potential for broken fingers for those who handle them	No effects
Domestic Dogs	Potential for poisoning of lost or stray dogs who consume large numbers of pellets	No potential for poisoning of dogs	No effects	No effects

Appendix 11. National Park Service acceptance of environmental report.

United States Department of the Interior

NATIONAL PARK SERVICE
Pacific West Region
600 Harrison Street, Suite 600
San Francisco, California 94107-1372

REPLY REFER TO:

L7617 (PGSO-PP) OCT 0 5 1999

Memorandum

To: Superintendent, Hawaii Volcanoes National Park

From: Regional Director, Pacific West Region

Subject: Environmental Compliance for a Test Broadcast of the Rodenticide *Ramik Green*

The revised *Finding of No Significant Impact* for this research phase of the park's long-term rodent control program is approved. To complete this particular compliance effort, the park should send notice of the decision to all individuals and organizations who received the supporting environmental assessment.

Martha R. Roosevelt

John J. Reynolds

Attachment

cc:
PISO, Barbano

QA-02

FINDING OF NO SIGNIFICANT IMPACT: TEST BROADCAST RODENTICIDES TO CONTROL RATS, HAWAI'I VOLCANOES NATIONAL PARK

PROPOSED ACTION

The National Park Service (Hawai'i Volcanoes National Park) proposes to test the rodenticide Ramik Green. This proposal is supported by the park's Resource Management Plan in projects HAVO-N-318 and HAVO-N-321. Ramik Green is a pelletized pesticide broadcast by hand or from a helicopter. It consists of a fish flavored grain bait and the pesticide diphacinone at 0.005%. Diphacinone is an anticoagulant which works by causing internal bleeding. Ramik Green will be applied at 20 pounds/acre starting fall, 1999 and reapplied every two to several months for a year, up to four times per year. The tests are designed to evaluate the efficacy of Ramik Green in controlling rats, possible toxicity to non-target species such as invertebrates and birds, and recovery of native plants and animals. The tests will first be conducted in a 10 acre study site in Kīpuka Kī and a 10 acre area in 'Ōla'a forest near Wright Road. Hand broadcasting will be used in the 10 acre sites. Ramik Green will then be tested in 50 acre sites in the same areas utilizing aerial broadcasting. Both test areas are within fenced units from which feral goats and pigs have been eliminated.

The tests will be conducted by US Geological Survey/Biological Resources Division scientists, in collaboration with US Department of Agriculture researchers. The research is needed to develop a tool for conservation managers in Hawai'i to control rats. Rats, especially the arboreal black rat, are known to be predatory on native bird eggs and young; they also consume large amounts of native plant seeds and invertebrates. All three species of rats in Hawai'i are introduced.

Ramik Green is currently registered for use as a rodenticide around dwellings, industrial, and agricultural buildings. Conservation agencies are attempting to develop a special local needs label for use in conservation areas. For registration of Ramik Green for use in conservation areas, the Environmental Protection Agency requires research on efficacy in controlling rats and effects on non-target organisms. A broadcast rodenticide is needed by conservation agencies because it will be much more cost-effective and practical in remote area than bait stations or snap trapping.

Other alternatives that were considered were use of diphacinone delivered in bait stations, use of snap traps, or no rat control. A more detailed description of the proposed action and alternatives is contained in the Environmental Assessment (EA): *Test Broadcast Rodenticide to Control Rats, Hawai'i Volcanoes National Park.*

WHY THE PROPOSED ACTION WILL NOT HAVE A SIGNIFICANT EFFECT ON THE ENVIRONMENT

The proposed action will not adversely affect cultural resources. The only potential impact on cultural resources is from digging small (4 x 8 inch) pit fall traps to sample invertebrates. These will be filled in after the study is completed. Archeological and historical resources are not abundant in either study site. An archeologist will accompany the researcher in locating the pit fall traps to assure that no features are disturbed. The purpose of conducting the tests is to monitor non target organisms to determine if there are unforeseen effects.

The use of diphacinone is not expected to pose a threat to human health and safety. All test areas will be signed, and are sparsely used by the visiting public. The lethal dose of diphacinone for humans is not definitively known. However, the therapeutic dose is approximately 34 pellets for an initial dose and 3-7 pellets per day as a maintenance dose. There is no danger to hunters because game animals are not present in the study sites. Likewise hunting dogs will not be affected, although stray dogs may be. A 30 pound dog would need to ingest 68 pellets to receive a lethal dose.

The proposed action and mitigation will not adversely affect natural resources. Baiting is expected to significantly reduce rat populations. Alien mongoose may be killed by the bait. They are very sensitive to diphacinone and are attracted to the bait in bait stations. No negative impacts on vegetation are anticipated. Most likely seedling establishment will be enhanced by rat control. The effects on soil are expected to be short-lived. Diphacinone is readily adsorbed by organic matter, and not expected to be mobile. It has a half-life of 35 days. Diphacinone is not soluble in water, there is no surface water in study areas, and the water table is located at great depths. Therefore, water resources will not be affected.

The endangered ʻIo or Hawaiian Hawk is not expected to be impacted by scavenging or preying of feeding on rats. Other rat control studies in natural areas in Hawaiʻi have demonstrated that dying rats contaminated by diphacinone are rapidly located by mammalian scavengers and tend to die in their nests. Moreover, ʻIo are almost exclusively diurnal and rats are almost exclusively nocturnal. ʻIo may also be exposed to diphacinone by preying on contaminated birds. Pesticide residues in several bird species will be analyzed in the study to check for this potential. Radio collared and banded ʻIo in and around the study sites will also be monitored during the study. The potential effects of diphacinone on ʻIo are discussed in greater detail in documents available from park files on Section 7 consultation with the US Fish and Wildlife Service (FWS). The Biological Opinion of the FWS is that the proposed study is not likely to jeopardize the continued existence of the ʻIo and that the control techniqes will be beneficial to other endangered species. The FWS has prescribed terms and conditions in the Biological Opinion requiring close monitoring for signs of toxicity in ʻIo, tracking rat movements, and necropsy of dead birds.

Invertebrates are apparently little affected by diphacinone. However, effects on selected invertebrates most likely to be affected will be assessed during the proposed study. The attractiveness of the baits and diphacinone effects on native picture-winged Drosophila, snails, and slugs will be evaluated by laboratory and/or field experiments by checking mortality or pesticide residues. Effects of baiting and rat control on invertebrate populations will also be evaluated by population monitoring. The potential for bioaccumulation in birds will be evaluated in the alien birds, kalij pheasant, northern cardinal, and leiothrix, which may be feeding on pellets or invertebrates feeding on pellets. These species will be collected and whole body analysis carried out.

Wilderness in ʻŌlaʻa will be affected by the presence of marked research plots and transects, temporary pit fall traps, use of a pesticide, and use of a helicopter to disperse Ramik Green pellets. Rat control, along with other restoration strategies such as pig control, were determined to be the minimum requirement for restoring the ecological integrity of wilderness, and the proposed research methodology was determined to be the minimum tool to carry this out. The short-term noise and other wilderness effects were deemed to be outweighed by the anticipated long-term benefits to the ecological

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integrity of wilderness.

PUBLIC REVIEW

The comment period was from July 28 to September 3, 1999. The draft EA was mailed to 78 individuals, organizations, and government agencies. These included bird hunting and pig hunting clubs, conservation organizations, natural resource management agencies, a wilderness protection group, Native Hawaiian community leaders and organizations, adjacent landowners, community groups, and biologists. Twenty-four responses were received by phone, email, or letter. Twenty respondents explicitly supported the proposal; three others found no problems with analysis or the project; no respondent objected to the proposal. A number of concerns were raised, especially from conservation biologists about potential effects on invertebrates and birds. Dr. Hampton Carson expressed concerns about diphacinone on endemic picture-wing Drosophila in Kipuka Ki. The research protocol was modified with Dr. Carson's concurrence to test bait attractiveness and effects of diphacinone on a laboratory population of picture-winged Drosophila, to supplement planned monitoring of bait attractiveness and population levels in the field during the course of the study. Dr. Grant Gerrish felt that the EA was deficient in information concerning potential secondary poisoning of native birds, especially the 'Io from preying on contaminated birds or rats. Victor Tanimoto, Entomologist, Department of Land and Natural Resources, felt there was some need to test soils and water. Steve Hurt, President Big Island Bird Hunter's Club, was concerned about effects of diphacinone on game birds such as turkeys and pleasants that may move from treated areas to hunting areas and effects on owls. The concerns of Dr. Gerrish and Mr. Hurt are addressed by changes in the EA to clarify testing for pesticide residues in birds. Dr. Loope, Dr. Duvall, and Mr. Anderson felt that study sites should be larger to evaluate efficacy of control and impacts on non target species, especially birds, or that the 10 acre study site stage should be by passed in favor of the 50 acre study areas to expedite the research. The 10 acre study site stage is not obligatory but the park and BRD principal investigators wanted to proceed cautiously and minimize potential impacts by starting in smaller research sites. Dr. Duvall also pointed out the seeds are included in the grain bait and have germinated under wet field conditions. Seeds that germinated included millet and buckwheat. Germination of seeds in Ramik Green pellets will be tested under optimal conditions in the greenhouse and under field conditions. The germinating species will be identified and the fate of the seedlings monitored under field conditions. The seven respondents who expressed concerns did not raise points which substantively altered the outcome of the environmental impact analysis.

IMPACT CATEGORY	MITIGATION MEASURE	RESPONSIBILITY
Human Health and Safety	Post warning signs; notify known user groups; Utilize lightly visited sites in park where no game animals are present or hunting is taking place	Chief of Resource Management/
Aesthetics	Remove all flagging, markers, traps at end of study	Chief of Resources Management
Cultural Resources	Pit fall traps will be dug when archeologist present to avoid resources; Holes will be filled in at end of study	Chief of Resources Management
Birds	Sample for pesticide residue in three alien bird species and potentially others, including native birds, if pesticide residues found; Monitor to in and around test sites using radio collared and banded birds; Monitor for dead birds in study areas and adjacent areas; Test for pesticide residue in any dead birds found	Chief of Resources Management
Invertebrates	Conduct laboratory tests on bait attractiveness and impacts of diphacinone on picture-wing Drosophila species; Conduct field test of bait attractiveness and pesticide residue in slugs and snails	Chief of Resources Management
Wilderness	Remove all markers and other evidence research when completed;Minimize helicopter use to bait drops only	Chief of Resources Management

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Plants	Potential chewing on experimental plants of endangered hau kuahiwi compensated by large number of outplantings; Germination of seeds in Ramik Green pellets to be tested under field conditions	Chief of Resources Management

DETERMINATION

Based on information contained in the Environmental Assessment as summarized above, the nature of the comments received during the public review period, and the mitigation measures to be taken, it is the determination of the National Park Service that the proposed action would not constitute a major federal action that would would significantly affect the human environment. Therefore, in compliance with the National Environmental Policy Act regulations, an Environmental Impact Statement is not required and the proposed action as detailed in the Environmental Assessment may be implemented immediately.

Submitted by _____ Date 9/3/99
Superintendent
Hawaii Volcanoes National Park

Approved by Martha R. Lucista Date 10/5/99
for Director
Pacific West Region

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Appendix 12. Ramik® Green label.

NOT FOR SALE
FOR RESEARCH PURPOSES ONLY

SAME FORMULATION AS: **RAMIK GREEN**, EPA Reg. No. 2393-498

This product was manufactured at the request of and with the cooperation of USDA/APHIS, Hilo, HI; Hawaii State Division of Forestry and Wildlife, Honolulu, HI; and National Park Service, U.S. Dept. of Interior, Hawaii Volcanoes National Park. This product will be used according to Hawaii Department of Agriculture Experimental Use Permit Numbers EUP-99-01 and EUP-99-02.

Active Ingredient:
Diphacinone (2-diphenylacetyl-1,3-indandione) 0.005%
Inert Ingredients:.....99.995%
Total.....100.000%

EPA Est. No. 61282-WI-1

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS
HAZARD TO HUMAN AND DOMESTIC ANIMALS
CAUTION: Keep away from humans, domestic animals and pets. If swallowed, this product may reduce the clotting ability of the blood and cause bleeding.
NOTE TO PHYSICIAN: If ingested, administer Vitamin K_1, intramuscularly or orally, as indicated in bishydroxycoumarin overdose. Repeat as necessary based on monitoring of prothrombin times.
IN ALL CASES OF HUMAN INGESTION IMMEDIATELY NOTIFY A PHYSICIAN.
ENVIRONMENTAL HAZARDS: This product is toxic to mammals and birds. Do not apply this product directly to water or to areas where surface water is present.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.
STORAGE: Store only in original closed container in a cool, dry place inaccessible to children and pets. Store separately from fertilizer and away from products with strong odors which may contaminate the bait and reduce acceptability. Spillage should be carefully swept up and collected for disposal.
PESTICIDE DISPOSAL: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.
PLASTIC CONTAINER DISPOSAL: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.
FIBER DRUMS WITH LINER DISPOSAL: Completely empty liner by shaking and tapping sides and bottom. Dispose of liner in a sanitary landfill or by incineration if allowed by state and local authorities. If fiber drum is contaminated, puncture and dispose of in same manner; otherwise, offer drum for recycling or reconditioning.

NOTICE OF WARRANTY: IT IS IMPOSSIBLE TO ELIMINATE ALL RISKS INHERENTLY ASSOCIATED WITH THIS PRODUCT. CROP INJURY, INEFFECTIVENESS, OR OTHER UNINTENDED CONSEQUENCES MAY RESULT BECAUSE OF SUCH FACTORS AS WEATHER CONDITIONS, PRESENCE OF OTHER MATERIALS, OR THE MANNER OF USE OR APPLICATION, ALL OF WHICH ARE BEYOND THE CONTROL OF HACO, THE MANUFACTURER OR SELLER. IN NO CASE SHALL HACO, THE MANUFACTURER OR SELLER BE LIABLE FOR CONSEQUENTIAL, SPECIAL OR INDIRECT DAMAGES RESULTING FROM THE USE OR HANDLING OF THIS PRODUCT. ALL SUCH RISKS SHALL BE ASSUMED BY THE BUYER.
EXCEPT AS EXPRESSLY PROVIDED HEREIN, HACO, THE MANUFACTURER OR SELLER MAKE NO WARRANTIES, GUARANTEES, OR REPRESENTATIONS OF ANY KIND, EITHER EXPRESS OR IMPLIED, OR BY USAGE OF TRADE, STATUTORY OR OTHERWISE, WITH REGARD TO THE PRODUCT SOLD, INCLUDING, BUT NOT LIMITED TO, MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, USE OR ELIGIBILITY OF THE PRODUCT FOR ANY PARTICULAR TRADE USAGE. BUYER'S OR USER'S EXCLUSIVE REMEDY, AND HACO'S, THE MANUFACTURER'S OR SELLER'S TOTAL LIABILITY, SHALL BE FOR DAMAGES NOT EXCEEDING THE COST OF THE PRODUCT.

FOR USE BY RESEARCHERS ONLY.

QA-02

HACO, INC.•P.O..BOX 7190•MADISON, WI 53707
NET CONTENTS: 50 pounds (22.68 Kg.)
CODE L/00

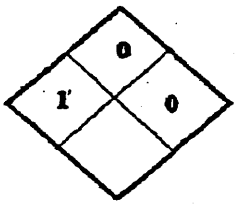
Appendix 13. Material safety data sheet.

RAMIK GREEN

MATERIAL SAFETY DATA SHEET

Product Name: RAMIK GREEN

EPA Registration Number: 2393-498



SECTION I

Manufacturer's Name: HACO, INC.
537 Atlas Avenue P.O. Box 7190
Madison, Wisconsin 53707

Emergency Phone Numbers: 608-221-6200 HACO, INC.
608-233-5039 Mid-Wisconsin Security
800-424-9300 CHEMTREC

Date Prepared: 1/30/97

SECTION II - Hazardous Ingredients/Identity Information

Specific Chemical Identity	OSHA PEL	ACGIH TLV	Other Limits Recommended	% Ingr.
Diphacinone (CAS No. 82-66-6)	N/A	N/A	N/A	0.005
Sodium Saccharin (CAS No. 128-44-9)	N/A	N/A	N/A	< 1.000
Inert Ingredients: (non-hazardous) Grain, flavoring, preservative	N/A	N/A	N/A	> 98.995

THIS PRODUCT CONTAINS THE FOLLOWING SUBSTANCE WHICH IS REGULATED UNDER SARA, TITLE III, SECTION 313: None

SECTION III - Physical/Chemical Characteristics

Boiling Point:	N/A
Specific Gravity (water = 1)	Bulk Density = 31-33 lb/ft^3
Vapor Pressure (mm Hg)	N/A
Vapor Density (air=1)	N/A
Melting Point:	N/A
Evaporation Rate (Butyl Acetate=1)	N/A
Solubility in Water:	Slightly soluble.
Appearance and Odor:	Green extruded pellets with fish odor.

SECTION IV - Fire and Explosion Hazard Data

Flash Point: N/A
Flammability Limits: UEL: N/A LEL: N/A
Extinguishing Media: Fog or water spray, foam, carbon dioxide, dry chemical

Special Fire Fighting Procedures: Potentially hazardous in severe fire.
Wear self-contained breathing apparatus. Heat from fire may cause decomposition with evolution of toxic and irritating fumes. If water is used as an extinguishing media, diking is required to keep contaminated water out of all water supplies.

Unusual Fire and Explosion Hazards: None.

N/A = Not Available

RAMIK GREEN

SECTION V - Reactivity Data

Stability: This is a stable material.
Conditions to avoid: None known.

Incompatibility (Materials to Avoid): None known.

Hazardous Decomposition Products: Aromatic decomposition products: Carbon Monoxide, Carbon Dioxide, Water.

Hazardous Polymerization: Does not occur.
Conditions to avoid: None known.

SECTION VI - Health Hazard Data

Routes of Entry: Inhalation? No.
Skin? No.
Ingestion? Yes.

Health Hazards (Acute and Chronic): Inhibition of formation of prothrombin and reduction of clotting of blood. Acute Oral LD_{50} = 2.3 mg/kg for Diphacinone Technical at 98% Active Ingredient. (Equivalent to 46,000 mg/kg of Ramik Green)

Carcinogenicity: NTP? Saccharin is a candidate chemical.
IARC Monographs? Saccharin is a candidate chemical.
OSHA Regulated? No.
Saccharin has been determined to cause cancer in laboratory animals.

Signs and symptoms of exposure: Normal reaction to anticoagulant, i.e. nose bleeding, bleeding gums.

Medical Conditions Generally Aggravated by Exposure: Bleeding and other conditions which may be aggravated by extended clotting time.

Emergency and First Aid Procedures: INGESTION: For large doses within preceding 2-3 hours induce vomiting by drinking 1 or 2 glasses of water and touching back of throat with finger. DO NOT induce vomiting or give anything by mouth to unconscious persons. Call Physician Immediately. Administration of Vitamin K_1 combined with blood transfusions, is indicated as in the case of hemorrhage caused by overdose of bishydroxycoumarin (Dicumarol).

SECTION VII - Precautions for Safe Handling and Use

Steps to Be Taken In Case Material Is Released or Spilled: Sweep up, place in container and seal.

Waste Disposal Method: If these wastes cannot be disposed of by use according to label instructions, (i.e. garbage dumps, etc.) contact your State Pesticide Agency.

Precautions to Be Taken In Handling and Storing: Store in original container in a cool dry area separately from fertilizer, feed, or foodstuffs and away from products with strong odors.

Other Precautions: Keep in area suitable for pesticide storage. Keep out of reach of children and domestic animals. Avoid cross-contamination with other pesticides.

SECTION VIII - Control Measures

Respiratory Protection (specify type): Not generally required.
Ventilation: Local Exhaust? Not generally required.
Mechanical (general)? Not generally required.
Special? Not generally required.
Other? Not generally required.

Protective Gloves: None
Eye Protection: None.
Other Protective Clothing or Equipment: Use clothing and equipment consistent with good pesticide handling and application procedures.

Work/Hygienic Practices: Wash thoroughly after handling product.

RAMIK GREEN

SECTION IX - California Addendum (Proposition 65) Safe Drinking Water and Toxic Enforcement Act of 1986

The following specific warnings are hereby given relative to substances that the State of California has identified as carcinogens and/or reproductive hazards under Proposition 65:

☒ WARNING: This product contains a chemical known to the State of California to cause cancer. (Sodium Saccharin)

☐ WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.

SECTION X - SARA TITLE III HAZARD CATEGORY:
For Reporting Under Sections 311 & 312

Immediate No Delayed Yes Fire No
Reactive No Sudden Release of Pressure No

SECTION XI - Shipping Information

D.O.T. Hazard Classification: Not D.O.T. Regulated.
Bill of Lading Description: Vermin Exterminators, NOI

All information contained in the Material Safety Data Sheet is furnished free of charge and is intended for your evaluation. In our opinion the information is, as of the date of this Material Safety Data Sheet, reliable, however, it is your responsibility to determine the suitability of the information for your use. You are advised not to construe the information as absolutely complete since additional information may be necessary or desirable when particular, exceptional or variable conditions or circumstances exist or because of applicable laws or government regulations. Therefore, you should use this information only as a supplement to other information gathered by you and you must make independent determinations of the suitability and completeness of the information from all sources to assure both proper use of the material described herein and the safety and health of employees. Accordingly, no guarantee expressed or implied is made by HACO, INC. as to the results to be obtained based upon your use of the information nor does HACO, INC. assume any liability arising out of your use of the information.

Appendix 14. End use product tracking form for Ramik® Green bait.

Date	Description	Quantity used (lb)	Quantity remain (lb)	Initials
8-Jul-1999	Received 2038 lb from HACCO, stored Bldg 216, BRD		2038.00	CFP
7-Oct-1999	Used 7680 baits Olaa TR 16, 1st half application #1	100	1938.00	CFP
7-Oct-1999	Used 30 baits for bait monitoring Olaa TR 16	0.4	1937.60	CFP
12-Oct-1999	Used 7680 baits Olaa TR 16, 2nd half application #1	100	1837.60	CFP
8-Dec-1999	Used 7680 baits Olaa TR 16, 1st half application #2	100	1737.60	CFP
8-Dec-1999	Used 20 baits for bait monitoring Olaa TR 16	0.25	1737.35	CFP
10-Dec-1999	Used 40 baits for freezer degradation study	0.5	1736.85	CFP
14-Dec-1999	Used 7680 baits Olaa TR 16, 2nd half application #2	100	1636.85	CFP
25-Jan-2000	Sent 50 baits (294 g) to Genesis for residue analysis	0.65	1636.20	CFP
27-Jan-2000	Used 7680 baits Kipuka Ki, 1st half application #1	100	1536.20	CFP
27-Jan-2000	Used 30 baits for bait monitoring Kipuka Ki	0.4	1535.80	CFP
1-Feb-2000	Used 7680 baits Kipuka Ki, 2nd half application #1	100	1435.80	CFP
8-Feb-2000	Used 7680 baits Olaa TR 16, 1st half application #3	100	1335.80	CFP
8-Feb-2000	Used 20 baits for bait monitoring Olaa TR 16	0.25	1335.55	CFP
11-Feb-2000	Used 50 baits for germination experiment	0.65	1334.90	CFP
14-Feb-2000	Used 7680 baits Olaa TR 16, 2nd half application #3	100	1234.90	CFP
12-Apr-2000	Used 7680 baits Olaa TR 16, 1st half application #4	100	1134.90	CFP
12-Apr-2000	Used 20 baits for bait monitoring Olaa TR 16	0.25	1134.65	CFP
17-Apr-2000	Used 7680 baits Olaa TR 16, 2nd half application #4	100	1034.65	CFP
14-Jun-2000	Used 7680 baits Olaa TR 16, 1st half application #5	100	934.65	CFP
14-Jun-2000	Used 20 baits for bait monitoring Olaa TR 16	0.25	934.40	CFP
19-Jun-2000	Used 7680 baits Olaa TR 16, 2nd half application #5	100	834.40	CFP
5-Jul-2000	Used 7680 baits Kipuka Ki, 1st half application #2	100	734.40	CFP
5-Jul-2000	Used 20 baits for bait monitoring Kipuka Ki	0.25	734.15	CFP
10-Jul-2000	Used 7680 baits Kipuka Ki, 2nd half application #2	100	634.15	CFP
21-Jul-2000	Gave 2 boxes (100 lb) to P. Dunlevy, APHIS	100	534.15	CFP
31-Aug-2000	Used 7680 baits Olaa TR 16, 1st half application #6	100	434.15	CFP
31-Aug-2000	Used 20 baits for bait monitoring Olaa TR 16	0.25	433.90	CFP
5-Sep-2000	Used 7680 baits Olaa TR 16, 2nd half application #6	100	333.90	CFP
25-Oct-2000	Used 7680 baits Kipuka Ki, 1st half application #3	100	233.90	CFP
25-Oct-2000	Used 20 baits for bait monitoring Kipuka Ki	0.25	233.65	CFP
30-Oct-2000	Used 7680 baits Kipuka Ki, 2nd half application #3	100	133.65	CFP
8-Nov-2000	Received 10 boxes from HACCO, stored Bldg 216		633.65	CFP
9-Nov-2000	Used 7680 baits Olaa TR 16, 1st half application #7	100	533.65	CFP
9-Nov-2000	Used 20 baits for bait monitoring Olaa TR 16	0.25	533.40	CFP
13-Nov-2000	Received 22 boxes from HACCO, stored Bldg 216		1633.40	CFP
15-Nov-2000	Used 7680 baits Olaa TR 16, 2nd half application #7	100	1533.40	CFP
18-Jan-2001	Used 7680 baits Olaa TR 16, 1st half application #8	100	1433.40	CFP
18-Jan-2001	Used 20 baits for bait monitoring Olaa TR 16	0.25	1433.15	CFP
22-Jan-2001	Used 7680 baits Olaa TR 16, 2nd half application #8	100	1333.15	CFP
19-Mar-2001	Used 7680 baits Olaa TR 16, 1st half application #9	100	1233.15	CFP
19-Mar-2001	Used 20 baits for bait monitoring Olaa TR 16	0.25	1232.90	CFP
23-Mar-2001	Used 7680 baits Olaa TR 16, 2nd half application #9	100	1132.90	CFP

19-Apr-2001	Removed 60 baits for measuring, then returned them		1132.90	DF
8-May-2001	Sent 72 baits to HACCO for analysis	0.95	1131.95	IS
24-May-2001	Used 7680 baits Olaa TR 16, 1st half application #10	100	1031.95	CFP
24-May-2001	Used 20 baits for bait monitoring Olaa TR 16	0.25	1031.70	CFP
29-May-2001	Used 7680 baits Olaa TR 16, 2nd half application #10	100	931.70	CFP
26-Jul-2001	Gave 20 baits to R. Sugihara, APHIS	0.25	931.45	CFP
23-Aug-2001	Used 7680 baits Olaa TR 16, 1st half application #11	100	831.45	CFP
23-Aug-2001	Used 20 baits for bait monitoring Olaa TR 16	0.25	831.20	CFP
27-Aug-2001	Used 7680 baits Olaa TR 16, 2nd half application #11	100	731.20	CFP
17-Dec-2001	Used 7680 baits Olaa TR 16, 1st half application #12	100	631.20	CFP
17-Dec-2001	Used 20 baits for bait monitoring Olaa TR 16	0.25	630.95	CFP
19-Dec-2001	Used 7680 baits Olaa TR 16, 2nd half application #12	100	530.95	CFP
8-Jan-2002	Sent 477.39 g to Genesis Lab for analysis	1.05	529.90	CFP
2-Apr-2002	Sent 378.757 g to Genesis Lab for analysis	0.84	529.06	CFP

Initials:
CFP = Charlotte Forbes Perry
DF = David Foote
IS = Ilana Stout

Appendix 15. HACCO certificates of analysis of Ramik® Green bait.

HACCO, INC.

Manufacturing Plant
110 Hopkins Drive
Randolph, WI 53956-1316
(920) 326-5141
FAX (920) 326-5135

Registration Office
P.O. Box 7190 (53707)
5900 Monona Dr.
Water Tower Place, Suite 200
Madison, WI 53716
(608) 221-6200 • FAX (608) 221-7380

CERTIFICATE OF ANALYSIS

Product: Ramik Green ¾"
Date of Manufacture: May 1999
Date Analyzed: May 1999

LOT NUMBER	% DIPHACINONE
125218	0.0051

Jodi Fields — 9-9-02
Jodi Fields — Date
Quality Control Lab Technician

MaryAnn Douglas — 9/9/02
MaryAnn Douglas — Date
Research Chemist

HACCO, INC.

Manufacturing Plant
110 Hopkins Drive
Randolph, WI 53956-1316
(920) 326-5141
FAX (920) 326-5135

Registration Office
P.O. Box 7190 (53707)
5900 Monona Dr.
Water Tower Place, Suite 200
Madison, WI 53716
(608) 221-6200 • FAX (608) 221-7380

CERTIFICATE OF ANALYSIS

Product: Ramik Green ¾"
Date of Manufacture: September 2000
Date Analyzed: September 2000

LOT NUMBER	% DIPHACINONE
144548	0.0051

Jodi Fields
Quality Control Lab Technician

9-9-02
Date

MaryAnn Douglas
Research Chemist

9/9/02
Date

HACCO, INC.

Manufacturing Plant
110 Hopkins Drive
Randolph, WI 53956-1316
(920) 326-5141
FAX (920) 326-5135

Registration Office
P.O. Box 7190 (53707)
5900 Monona Dr.
Water Tower Place, Suite 200
Madison, WI 53716
(608) 221-6200 • FAX (608) 221-7380

CERTIFICATE OF ANALYSIS

Product: Ramik Green ¾"
Date of Manufacture: September 2000
Date Analyzed: May 2002

LOT NUMBER	% DIPHACINONE
144548	0.0044

Jodi Fields
Quality Control Lab Technician

9-9-02
Date

MaryAnn Douglas
Research Chemist

9/9/02
Date

Appendix 16. Malkov and Mach (2002), Genesis Laboratories report.

FINAL REPORT

STUDY TITLE

Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005 % Diphacinone Bait

DATA REQUIREMENTS

Subdivision E: Hazard Evaluation Wildlife and Aquatic Organisms
OPPTS 850.2500, 71-5

AUTHORS

Vadim Malkov
Jeff J. Mach, Study Director

PERFORMING LABORATORY

Genesis Laboratories, Inc.
10122 N.E. Frontage Road
Wellington, CO 80549

STUDY COMPLETION

July 25, 2002

STUDY NUMBER

00005

SPONSOR

U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
P.O. Box 44/Bldg. 344
Hawaii Volcanoes National Park, HI 96718

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

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

David Foote

17 July 2002

David Foote
U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study contained herein, 00005, was conducted in accordance with requirements of Title 40, Code of Federal Regulations, Part 160, Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs.

Study Director: _____ 7/25/02
Jeff J. Mach Date
Genesis Laboratories, Inc.

Sponsor Representative: _____ 17 July 2002
David Foote Date
U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

Sponsor Submitter: _____ 17 July 2002
David Foote Date
U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

QUALITY ASSURANCE STATEMENT

The study, 00005, was monitored by the Quality Assurance Unit of Genesis Laboratories, Inc. In order to evaluate the study 00005 in terms of compliance with Title 40, Part 160 of the Code of Federal Regulations, Good Laboratory Practice Standards, the study was inspected at different critical phases. The dates of inspections are listed below. The report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Inspection Phase	Inspection Date	Date Submitted to Study Director	Date Submitted to Management
Protocol	01/25/00	01/25/00	01/26/00
Method Validation and Concentration Verification	03/27/00	03/27/00	03/31/00
Raw Data	04/13/00, 04/14/00	04/14/00	04/19/00
Removal of Livers	04/27/00	04/28/00	04/28/00
Liver Extraction	05/03/00	05/03/00	05/08/00
Raw Data Review	05/24/00	05/24/00	06/08/00
Concentration Verification Analysis	05/30/01	05/30/01	06/01/01
Bait Extraction for Analysis	01/16/02	01/17/02	01/17/02
Liver Removal and Extraction	01/17/02, 01/18/02	01/24/02	01/24/02
Bait Extraction Data	01/29/02	01/29/02	01/30/02
Review of Analytical Data	02/26/02	02/26/02	02/27/02
Draft Report	04/04/02	04/04/02	04/05/02
Final Report	06/06/02	06/06/02	06/12/02

Karen L. March
Quality Assurance Unit Manager

25 July 02
Date

GENESIS LABORATORIES PERSONNEL INVOLVED IN STUDY 00005

PERSONNEL	JOB TITLE
Jeff J. Mach	Study Director (09/19/00 – present)
Vadim Malkov	Chemist
Chris Gates	Laboratory Technician
John Baroch	Laboratory Technician
Jeff Borchert	Laboratory Technician
Valerie L. Fuhrman	Study Director (03/24/00 – 09/19/00)
Ronald J. Harkrader	Study Director (02/08/00 – 03/24/00)

STATEMENT OF STUDY INTEGRITY

There were no known circumstances that may have adversely affected the quality or integrity of the data.

Study Director: Jeff J. Mach _____ Date 7/25/02

LOCATION OF RAW DATA, TEST SUBSTANCE SAMPLES, AND FINAL REPORT

All raw data, test substance samples relating to the study, a copy of the original final report, all written communications between Genesis Laboratories, Inc. and the sponsor, and Standard Operating Procedures (SOPs) are kept in the archives of Genesis Laboratories, Incorporated.

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EXECUTIVE SUMMARY

STUDY TITLE:	Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005 % Diphacinone Bait
STUDY DIRECTOR:	Jeff J. Mach
STUDY INITIATION:	02/08/00
EXPERIMENTAL START DATE:	04/04/00
EXPERIMENTAL TERMINATION:	02/05/02
STUDY COMPLETION:	07/25/02
STUDY SPONSOR:	U.S.G.S. Biological Resources Division Pacific Island Ecosystem Research Center Kilauea Field Station P.O. Box 44/Bldg. 344 Hawaii Volcanoes National Park, HI 96718
PERFORMING LABORATORY:	Genesis Laboratories, Inc. 10122 N.E. Frontage Road Wellington, CO 80549
GENESIS LABORATORY STUDY NUMBER:	00005
TEST SUBSTANCE:	Ramik Green Bait Pellets
INVESTIGATED TISSUE:	Animal Liver
ACTIVE INGREDIENT (CAS #):	Diphacinone (82-66-6)

RESULTS: The concentration of diphacinone in four shipments of the Ramik Green Bait was determined to be 46.4 ± 1.2 ppm (00-TS-02), 49.1 ± 1.2 ppm (01-TS-08), 40.3 ± 2.2 ppm (02-TS-01), and 52.0 ± 1.1 ppm (02-TS-06). Three shipments of birds and other small animals were analyzed for presence and content of the residual diphacinone in their livers. The analysis of 20 birds from the initial lot received 03/29/00 showed a presence of diphacinone in 4 birds: #37, 44, 47 (Red-Billed Leothrix), and #33 (Kalij Pheasant) in the amounts 0.66, 0.33, 0.70, and 0.09 ppm, respectively. The second analyzed lot consisted of 11 birds, which were logged in as test substances 02-TS-02 A – K. Residual diphacinone was found in 7 birds from this lot, such as: #6, 8, 15 (Red-Billed Leothrix), #11, 16 (Northern Cardinal), and #24, 25 (Kalij Pheasant) in the amounts 1.25, 1.34, 0.74, 0.13, 0.08, 0.12, and 0.18 ppm, respectively. The analysis of the third lot (02-TS-07 A – C) discovered a presence of the analyte in every liver: #24 (Northern Cardinal) – 0.39 ppm, #36, 37 (House Mouse) – 2.39 and 1.75 ppm, respectively.

INTRODUCTION

The purpose of this study was to determine and verify concentration of the diphacinone bait test substance (Ramik Green Bait Pellets), which contains the active ingredient – diphacinone (CAS # 82-66-6), and determine the concentrations of test substance in animal species exposed to 0.005% diphacinone bait during the test period, using Good Laboratory Practice Standards (GLP). The study initiation date was February 8, 2000 and the experimental termination date was February 5, 2002.

TEST SUBSTANCE DESCRIPTION AND IDENTIFICATION

Genesis Laboratories, Inc. (GL) received from the Sponsor four parts of the Ramik Green Bait Pellets, packed in plastic bags (ZipLock bag). The first part (00-TS-02, Lot #125128) was received on January 28, 2000, and contained approximately 300 g of the test substance. The second part (01-TS-08, Lot #144548) was received on May 5, 2001, and contained approximately 550 g of the test substance. The last two parts were received on January 9, 2002 (02-TS-01, Lot #144548), and January 31, 2002 (02-TS-06, Lot #144548), and contained approximately 500 g of the test substance each. The active ingredient for each part of the test substance was diphacinone, 0.005%. All the bait was stored in designated storage.

Also, the Sponsor sent, and Genesis Laboratories, Inc. received three shipments of frozen animal carcasses stored in ZipLock bags. The first shipment was received on March 29, 2000 and contained 20 numbered birds' carcasses, which were used under their numbers. Other two parts were received on January 9, 2002 (02-TS-02 A - K), and January 31, 2002 (02-TS-07 A - C). All the received carcasses were stored in a freezer.

Every shipment was accompanied with a chain of custody form.

TEST METHODS AND MATERIALS

DIPHACINONE CONCENTRATION VERIFICATION IN BAIT

The diphacinone concentration in the Ramik Green Bait Pellets was determined using the validated method GL # 82-66-6-4, which included extraction of the active ingredient by refluxing a ground bait sample with 50.0 mL of the HPLC mobile phase. Then the analyte concentration was determined using a high performance liquid chromatography (HPLC) that employed a reversed phase column and UV detection. The method was validated for the concentration range 2.39 - 19.1 µg/mL with the following parameters: Mean R^2 = 0.99970 ± 0.00023, Fortified Sample Recovery = 103 ± 7.5 %, Sample CV = 7.27 %, Limit of Detection (LOD) was determined as a triple standard deviation for six consecutive injections of the lowest standard (2.39 µg/mL), and was 0.10 µg/mL. Limit of Quantitation (LOQ) was determined as the standard deviation multiplied by 10, and was 0.34 µg/mL. LOD and LOQ were determined to be 1.00 and 3.40 ppm for 5-g sample, respectively. HPLC mobile phase and extraction solution consisted of 55 % acetonitrile and 45 % aqueous tetrabutylammonium phosphate (IPC A reagent) with pH = 6.9 ± 0.1.

DETERMINATION OF DIPHACINONE IN ANIMAL LIVER

The residual concentration of diphacinone in the liver was determined by the validated method GL # 86-66-6-5, which consisted of diphacinone extraction, using a common extraction procedure, and then determination of the active ingredient concentration by a high performance liquid

for the concentration range 0.07 - 0.96 µg/mL with the following parameters: Mean R^2 = 0.99106 ± 0.00313, Fortified Blank Recovery = 81 ± 4 %, Sample CV = 4.9 %, LOD = 0.06 µg/mL, and LOQ = 0.16 µg/mL. Pure acetonitrile was used for the primary extraction of diphacinone (agitated in the Wrist Action Shaker), then the collected solution was vaporized under vacuum to dryness and the residue was dissolved in 3.0 mL of the HPLC mobile phase. For an average liver sample mass of 0.5 g, the LOQ was determined to be 0.96 ppm (LOQ = 0.16 µg/mL x 3 mL / 0.5 g).

RESULTS AND DISCUSSION

DIPHACINONE CONCENTRATION VERIFICATION IN BAIT

Every shipment of received Ramik Green Bait was analyzed for verification of diphacinone concentration. The concentration was found to be in the acceptable range for the test substances 00-TS-02, 01-TS-08, and 02-TS-06 (98.3 ± 5.6 % recovery). The concentration of diphacinone in the test substance 02-TS-01 was determined to be 80.6 % of theoretical that is slightly low. Detailed results of the analyses are presented in the Appendix II, Table 1.

DETERMINATION OF DIPHACINONE IN ANIMAL LIVER

Total number of animal livers analyzed for diphacinone was 34 samples. Among the liver samples were 32 samples from birds and 2 samples from mice. Twelve birds' and both mice livers contained residual amounts of diphacinone, and no diphacinone was found in 20 liver samples of the birds. The concentration of diphacinone in the birds from the first shipment - #37, 44, 47 (Red-Billed Leothrix), and #33 (Kalij Pheasant) was determined as 0.66, 0.33, 0.70, and 0.09 (mean) ppm, respectively. A presence of residual diphacinone was found in 4 birds from the second shipment (02-TS-02), such as: #15 (Red-Billed Leothrix), #11, 16 (Northern Cardinal), and #24, 25 (Kalij Pheasant) in the amounts 0.74, 0.13, 0.08, 0.12, and 0.18 ppm, respectively. The analysis of the third shipment (02-TS-07) discovered the similar level of the analyte concentration in the liver of bird #24 (Northern Cardinal) – 0.39 ppm. All the mentioned above concentrations were lower than the average LOQ (0.96 ppm); therefore the amount of diphacinone was not reliably quantifiable, and has to be concluded as a presence of the contaminant.

As for the other birds from the test group 02-TS-02 - #6, 8 (Red-Billed Leothrix) - 1.25 (mean) and 1.34 ppm, as well as for both mice samples (02-TS-07B,C - #36, 37 House Mouse) – 2.39 and 1.75 ppm, the concentrations were higher than LOQ, hence quantifiable, and the samples are considered to be obviously contaminated with diphacinone.

Detailed results of the analyses are presented in the Appendix II, Table 2.

CONCLUSIONS

The concentration of diphacinone in the bait was verified and determined to be in generally acceptable range (80 – 120% recovery). Thirty four samples of animal livers were analyzed and no diphacinone was found in 20 liver samples of the birds. The presence of the active ingredient was found in 14 liver samples – 12 birds and 2 mice, and among them 4 samples (2 birds and 2 mice) had the reliably quantifiable concentration of diphacinone (higher than the Limit of Quantitation).

APPENDIX I

PROTOCOL,

AMENDMENTS AND DEVIATIONS

PROTOCOL

STUDY TITLE

Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005 % Diphacinone Bait

DATA REQUIREMENTS

Subdivision E: Hazard Evaluation Wildlife and Aquatic Organisms
OPPTS 850.2500
71-5

AUTHOR

Ronald J. Harkrader, Ph.D.
Study Director

PERFORMING LABORATORY

Genesis Laboratories, Inc.
10122 N.E. Frontage Road
Wellington, CO 80549

STUDY NUMBER

00005

SPONSOR

U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
P.O. Box 44/Bldg. 344
Hawaii Volcanoes National Park, Hawaii 96718

PROTOCOL ACCEPTANCE

Product:	0.005% Diphacinone Bait
EPA Registration Number:	None Assigned
Active Ingredient:	Diphacinone
Study Number:	00005

LABORATORY PERFORMING ALL EXPERIMENTATION IN THIS PROTOCOL:

Genesis Laboratories, Inc.
10122 N.E. Frontage Road
Wellington, Colorado 80549

Robert. Troup, M.S. — Date 2/8/00
Technical Manager
Genesis Laboratories, Inc.

Ronald J. Harkrader, Ph.D. — Date 2/08/2000
Study Director
Genesis Laboratories, Inc.

PROTOCOL ACCEPTANCE, SPONSOR

Gerald Lindsey — Date 2-3-00
U.S.G.S. Biological Resource Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, Hawaii 96718

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INTRODUCTION AND PURPOSE

The purpose of this study is to determine and verify the concentration of the diphacinone bait test substance, which contains the active ingredient, diphacinone (CAS NO.: 82-66-6) and determine the concentrations of test substance in avian species exposed to 0.005% diphacinone bait during the test period, using Good Laboratory Practice Standards (GLP) in support of the registration of the product under 40 CFR 158.145. The manufactured lot number and formula number will be specified in the final report.

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

It is proposed that this GLP study be conducted from January 27, 2000 to November 1, 2000. The actual dates will be specified in the final report.

TEST SUBSTANCE DESCRIPTION

The test substance shall be one (1) lot of 0.005% by weight Diphacinone Bait. It will be supplied by the Sponsor. It is the responsibility of the Sponsor to provide approximately 100 g of the test substance. The test substance must be representative of the commercial lots or batches.

The test substance samples shall be labeled with product name, EPA registration number or CAS number, lot number if available, storage requirements, expiry date of that lot, if known, and date of synthesis or fabrication. A MSDS for the test substance must accompany the sample.

It is also understood that the Sponsor must provide analytical standards or designate Genesis Laboratories, Inc. as the party in charge of obtaining the appropriate analytical standards. The accompanying documents must include purity, lot number, storage requirements, and an expiry date.

As referenced in 40 CFR 160.105, the Sponsor is responsible for determining and documenting the identity, strength, purity and composition, certificate of analysis, or other characteristics which will appropriately define the test substance before its use in this GLP study. Methods of synthesis, fabrication, or derivation of the test substance shall be documented by the Sponsor and the location of such documentation shall be specified.

Any unused product will be returned to the Sponsor for proper disposal or recycling. The Sponsor must prepare accordingly.

SUMMARY OF EXPERIMENTATION TO BE PERFORMED

Pesticide Assessment Guidelines
Subdivision E: Hazard Evaluation Wildlife and Aquatic Organisms

CONCENTRATION VERIFICATION

The diphacinone bait test substance used in the field study will be analyzed for diphacinone concentration to determine the actual concentration of the active ingredient in the test substance.

The diphacinone concentration in the bait will be analyzed using High Performance Liquid Chromatography (HPLC). After the extraction of the diphacinone bait in a suitable solvent such as methanol, the samples will be shaken and then centrifuged. The supernatant will be decanted from the solid and transferred to Class A volumetric glassware. The extraction will be repeated one additional time. The supernatant will be transferred to the same Class A volumetric glassware and brought to volume with methanol. A sample will be filtered and analyzed by HPLC using diphacinone analytical standards to compare the sample concentration to a known concentration. All methods will be validated according to the current version of Genesis Laboratories, Inc. Standard Operating Procedure (SOP) AN-2 and the details of the method used, including calculations, will be reported in the final report.

Evaluation of Avian Species

Avian Species which are recovered during the diphacinone field testing period which are dead will be shipped frozen to Genesis Laboratories, Inc. for evaluation of diphacinone related deaths. Genesis Laboratories will develop a method for the analysis of liver and other soft tissues removed from the avian species to determine the concentration of diphacinone in the avian species. The avian tissues tested for the active ingredient will be reported in the final report.

All avian carcasses will be weighed prior to analysis of select tissues from the bird. The bird will then be thawed and selected samples from the bird will be removed and subjected to chemical extraction, and concentration techniques to isolate diphacinone from the tissues. All methods and their appropriate validation will be made according to the current version of Genesis Laboratories, Inc. SOP AN-2. A full report of the analytical methods used for residue diphacinone in avian tissues will be made in the final report.

The final report will include a summary of the avian species tested, the concentration of diphacinone residue found in the bird and a summary of the limits of the method used to

determine the residue in the animal including the limit of detection and limit of quantitation.

STATISTICAL METHODS

The active ingredient concentration found in the bait will be determined by interpolation of a linear regression relationship generated from analytical response factors. The sample values will be adjusted for individual sample weights and purity of the analytical standard. No adjustment will be made for method recovery (bias). The concentration of the active ingredient will be reported as the mean (w/w) percent plus or minus one standard deviation unit.

The diphacinone residue in the avian species will be reported based upon the sample and/or species weight. The sample values will be adjusted for individual weights and the purity of the analytical standard. No adjustment will be made for residue analysis method recovery (bias). The concentration of diphacinone residue will be reported as the weight percent. All evaluations will include an evaluation of the limit of detection and limit of quantitation for the analysis method. All calculations and statistical methods will be detailed in the final report.

AMENDMENTS TO THE PROTOCOL

All protocol amendments will be expressed in writing and will be signed and dated by the Study Director. Amendments will usually be issued prior to the initiation of protocol change. However, when a change is required without sufficient time to issue a written amendment, the change may be communicated verbally by the Study Director to the Sponsor. The verbal notice will be followed with a written amendment as soon as possible. In this case, the effected date of the written amendment will be the date of the verbal change. The procedure is detailed in the current revision of SOP SI-3. Copies of all signed amendments will be appended to the final report.

DATA RECORD KEEPING

All original data generated in support of this GLP study will be documented according to Genesis Laboratories Standard Operating Procedures. All data will be verified and maintained in folders in the raw data file. Other comments, descriptions, calculations, correspondence, and other study related documents will also be placed in the raw data file.

Upon completion of the study, a complete study report, including copies of representative raw data, will be submitted to the Sponsor. A complete and accurate study file, including all original raw data will be archived at Genesis Laboratories for permanent storage.

QUALITY ASSURANCE

The study will be monitored by an independent Quality Assurance Unit (QAU). All raw data and the final report will be audited to ensure compliance with Good Laboratory Practice Standards. An independent QAU will verify all data for accuracy and adherence to this protocol.

GLP STATEMENT

This GLP study will be conducted in accordance with the Regulations of Good Laboratory Practice Standards 40 CFR 160, set forth in the Genesis Laboratories Standard Operating Procedures. These SOPS are available for inspection.

SAFETY AND HEALTH

All laboratory personnel have been trained according to OSHA regulations and practice these guidelines throughout the course of experimentation. The Sponsor, however, must provide all pertinent Material Safety Data Sheets for the test substance and all active ingredients in the study. The Material Safety Data Sheet will be available to all personnel involved in the study.

FINAL REPORT

The Study Director at the conclusion of the analysis will prepare a draft report. After receipt of the Sponsor's comments, a final report will be prepared by the Study Director. The report will include, but not necessarily be limited to, the following.

A. Name and address of the facility performing the study and the dates on which the study was in initiated, completed, terminated, or discontinued.

B. Objectives and procedures stated in the approved protocol, including any changes to the original protocol.

C. Statistical methods employed for analyzing data.

D. The test, control, and reference substances identified by name, chemical abstract service (CAS) number, code number, strength, purity, and composition or other appropriate characteristics.

E. Stability and, when relevant to the conduct of the study, the solubility of the test, control, and reference substances under the conditions of administration.

F. A description of the methods used.

G. All deviations from the protocol.

H. A description of all circumstances that may have affected the quality or integrity of the data.

I. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

J. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

K. The signed and dated reports of each of the individual scientist or other professionals involved in the study, including each person who, at the request or direction of the testing facility Sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

L. The location where all specimens, raw data, and the final report are to be stored.

M. The statement prepared and signed by the Quality Assurance Unit.

N. A statement signed and dated by the Study Director indicating that the study was conducted in compliance with 40 CFR 160, Good Laboratory Practice Standards, and all parts of the study done outside compliance with these regulations will also be reported.

O. A confidentiality statement worded by and to be signed by the Sponsor.

Genesis Laboratories, Inc. Form SI-3A 11/01/96

PROTOCOL AMENDMENT NUMBER 1

1. STUDY NUMBER
00005

2. STUDY TITLE
Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005% Diphacinone Bait

3. SPONSOR
U.S.G.G. Biological Resources Division

4. AMENDMENT
The Protocol lists Ronald J. Harkrader as the Study the Director. The Study Director was changed to Valerie L. Fuhrman on March 24, 2000.

5. REASON FOR THE AMENDMENT
Ronald J. Harkrader resigned in March.

6. EFFECT OF THE AMENDMENT
None.

7. SPONSOR/SPONSOR REPRESENTATIVE INFORMED: By: VLF Date: 3/24/00
Method: (Verbal)/Written/Fax/E-mail/ _____

8. APPROVAL OF AMENDMENT NUMBER 1

Valerie L Fuhrman — Study Director — 3/24/00 Date

Genesis Management — 3/24/00 Date

Gerald O Lindsey — Sponsor/Sponsor Representative — 4/4/00 Date

Distribution: Sponsor/QA Officer/Technical Manager/Technicians N/A

Genesis Laboratories, Inc. SI-3A 11/01/96

Protocol Amendment #2

1. **Study Number** 00005

2. **Study Title** Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005% Diphacinone Bait

3. **Study Sponsor** U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
P.O. Box 44/Bldg. 344
Hawaii Volcanoes National Park, Hawaii 96718

4. **Amendment** Protocol amendment #1 changed the Study Director to Valerie Fuhrman. Ms. Fuhrman has left Genesis Laboratories, Inc. Jeff J. Mach will be the new Study Director as of September 19, 2000.

5. **Reason for Amendment** Ms. Fuhrman has resigned.

6. **Effect of Amendment** None.

7. **Sponsor/Sponsor Representative Informed**

by Verbal/Written/Fax/E-mail Date

8. **Approval of Amendment #1 2** 1/17/02

① Sponsor
~~Study Director~~ Date 5/1/01

Genesis Management Date 5/7/1

② ~~Sponsor/Sponsor Representative~~ Study Director Date 5/7/01

IACUC Representative NA Date NA

① Sponsor signed on wrong line. Changed for clarity 5/7/01

Genesis Laboratories, Inc. Form SI-3.03B 5/16/01

PROTOCOL DEVIATION #1

1. **STUDY NUMBER** 00005

2. **STUDY TITLE** Secondary Toxicity Examination of Avian Species on Fields Treated With 0.005% Diphacinone Bait

3. **SPONSOR** U.S.G.S. Biological Resources Division
Pacific Island Ecosystem Research Center
Kilauea Field Station
P.O. Box 44/Bldg. 344
Hawaii Volcanoes National Park, Hawaii 96718

4. **DEVIATION FROM THE PROTOCOL** List section (s) affected: Concentration Verification

The extraction method was not conducted according to the protocol.

5. **REASON FOR THE DEVIATION**

The method that is described in the protocol did not perform to the level that we required. Therefore, another method was validated to conduct the analysis.

6. **EFFECT OF THE DEVIATION**

No effect.

7. **APPROVAL OF DEVIATION**

Study Director	Date 1/17/02
Genesis Management	Date 1/17/02
Sponsor/Sponsor Representative	Date 7/17/02
IACUC Representative	Date

Genesis Laboratories, Inc. Form SI-3.03B 05/16/01

PROTOCOL DEVIATION

NUMBER 2

1. **Study Number:** 00005

2. **Study Title:** Secondary Toxicity Examination of Avian Species on Field Treated with 0.005 % Diphacinone Bait

3. **Sponsor:** Company: USGS Biological Resources Division Pacific Island Ecosystem Research Center Kilauea Field Station

   Contact: Charlotte M. Forbes

4. **Deviation from the Protocol:** Section affected: TEST SUBSTANCE DESCRIPTION

   The provided test substance was not from the same lot. The first shipment (01/26/00) was from the lot # 125128; all three next shipments were from the lot # 144548.

5. **Reason for the Deviation:**

   Since the study continued for 2 years, fresh bait was needed.

6. **Effect of the Deviation:**

   No adverse effect, because the main part of the analyzed test substance belonged to the same lot.

7. **Deviation Approval:**

Study Director — Date: 4/9/02

Genesis Management — Date: 4/9/02

Sponsor/Sponsor Representative — Date: 4/17/02

IACUC Representative (if required) — Date

APPENDIX II

RAW DATA SUMMARIES

DIPHACINONE CONCENTRATION VERIFICATION IN BAIT

Sample Extraction Procedure

A representative part of Ramik Green Bait was ground in the UDY Mill. Aliquots of the ground bait were weighed into tared 250 mL Erlenmeyer flasks in amount of 5.00 ± 0.10 gram each. Exactly 50.0 mL of the extraction solvent (consisted of 55% acetonitrile with 45% aqueous tetrabutylammonium phosphate (IPC A reagent) with pH = 6.9 ± 0.1) was added to each sample. Every sample was refluxed for 45 minutes. After refluxing, all samples were allowed to cool to room temperature, and an aliquot of each extract was filtered into a HPLC vial through 0.20 um syringe filter.

Sample Analysis

The HPLC system consisted of a Waters 515 HPLC pump, Waters 746 Data Module, Waters 486 Tunable Absorbance Detector, and a 717 Plus Autosampler. The column used for this analysis was a Keystone ODS/H column (4.6 mm x 250 mm, 5µm particle size). A Rheodyne Prefilter with a 10 µm steel frit was also used. The results of the concentration verification analyses are presented in Table 1 below. Examples of the chromatograms for the diphacinone analytical standard and the bait sample are presented in the Appendix III (-a, -b).

Table 1. Diphacinone Concentration Verification

Test Substance ID / Lot Number	Date of Analysis	Diphacinone Concentration per Extracted Sample, ppm	Mean Diphacinone Concentration ± Std. Dev., ppm	Recovery, %	CV, %
00-TS-02 / 125128	04/04/00	47.8, 44.8, 46.7, 46.3 [1]	46.4 ± 1.2	92.8	2.59
01-TS-08 / 144548	05/31/01	47.0, 48.9, 49.6, 48.9, 50.4, 49.7	49.1 ± 1.2	98.2	2.44
02-TS-01 / 144548	01/16/02	43.1, 40.5, 42.1, 39.2, 39.9, 36.8	40.3 ± 2.2	80.6	5.46
02-TS-06 / 144548	02/04/02	51.8, 51.0, 53.6, 51.5 [1]	52.0 ± 1.1	104	2.12

[1] – two more samples were used with spike to verify the extraction procedure performance.

DETERMINATION OF DIPHACINONE IN LIVER

Sample Preparation Procedure

Livers were taken from the carcasses and placed into tared centrifuge tubes, and then were cut with a scalpel (or with scissors) in the same centrifuge tubes, if the liver weight was less than approximately 1.3 ± 0.1gram. Otherwise, 1.00 ± 0.1-gram aliquot of the liver was taken and weighed into another tared tube and was minced there with a scalpel or scissors. The instrument used to mince the liver was rinsed in triplicate with acetonitrile after each use and the rinses were added to the same tube. Approximately 15 mL of acetonitrile was added to the tubes. The samples were shaken in the Wrist Action Shaker at maximum speed for 10 minutes and centrifuged at 60% of power for approximately 5 minutes. The supernatants were decanted into 125-mL round-bottom flasks. The extraction was repeated 2 additional times with fresh aliquots of acetonitrile. The collected supernatants were evaporated on the Rotavapor at approximately 35°C to dryness, and the residues were dissolved in 3.0 mL (Class A pipettes) of the mobile phase and sonicated for approximately 3 minutes at ambient

approximately 3 minutes at ambient temperature. An aliquot of each sample was filtered through a 0.20 um syringe filter into an HPLC vial.

Sample Analysis

The analyses were conducted using the same instrument (HPLC system) and column as for the bait analysis. The results of the determination of diphacinone concentration in animal livers are presented in Table 2 below. Examples of the chromatograms for the corresponding diphacinone analytical standard and the liver samples are presented in the Appendix III (-c through -e)

Table 2. Results of the Animal Liver Analyses

Date Received	Animal Number[1]	Common Name [1]	Genesis Laboratories ID	Liver Sample Mass, g	Diphacinone Concentration, ppm
03/29/00	11	Japanese White Eye	NA	0.20	ND
	12	Japanese White Eye	NA	0.35	ND
	15	N.A. Cardinal	NA	0.33	ND
	16	Red-Billed Leothrix	NA	0.28	ND
	17	Red-Billed Leothrix	NA	0.27	ND
	29	Kalij Pheasant	NA	1.02, 0.95	ND
	30	Kalij Pheasant	NA	1.01, 0.98	ND
	31	Kalij Pheasant	NA	0.92,1.03	ND
	32	Kalij Pheasant	NA	0.91, 0.94	ND
	33	Kalij Pheasant	NA	1.00, 0.99	0.06, 0.11
	36	Japanese White Eye	NA	0.37	ND
	37	Red-Billed Leothrix	NA	0.24	0.66
	40	Japanese White Eye	NA	0.40	ND
	41	N.A. Cardinal	NA	0.25	ND
	42	N.A. Cardinal	NA	0.35	ND
	43	N.A. Cardinal	NA	0.25	ND
	44	Red-Billed Leothrix	NA	0.31	0.33
	46	N.A. Cardinal	NA	0.24	ND
	47	Red-Billed Leothrix	NA	0.28	0.70
	48	Red-Billed Leothrix	NA	0.42	ND
01/09/02	3	Japanese White Eye	02-TS-02D	0.67	ND
	4	Japanese White Eye	02-TS-02E	0.67	ND
	5	Japanese White Eye	02-TS-02F	0.46	ND
	6	Red-Billed Leothrix	02-TS-02G	1.16	1.23, 1.27[2]
	8	Red-Billed Leothrix	02-TS-02H	1.17	1.34
	11	Northern Cardinal (M)	02-TS-02J	1.10	0.13
	15	Red-Billed Leothrix	02-TS-02I	0.88	0.74
	16	Northern Cardinal (F)	02-TS-02K	1.26	0.08
	18	Northern Cardinal (F)	02-TS-02C	0.80	ND
	24	Kalij Pheasant (M)	02-TS-02B	1.37	0.12
	25	Kalij Pheasant (F)	02-TS-02A	1.21	0.18
01/31/02	24	Northern Cardinal	02-TS-07A	0.74	0.39
	36	House Mouse	02-TS-07B	0.35	2.39
	37	House Mouse	02-TS-07C	0.32	1.75

ND = Not Detected

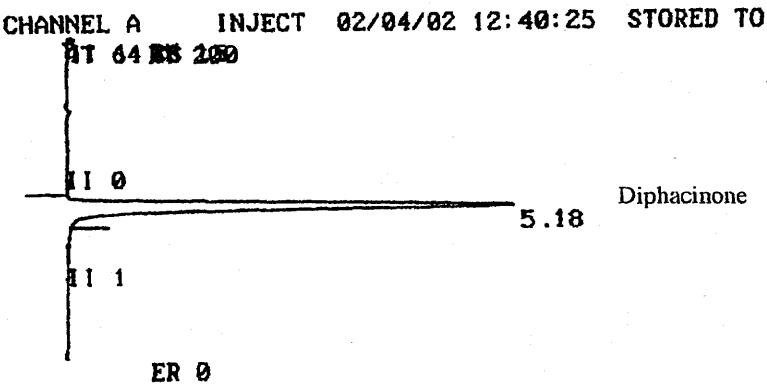
[1] - according to the chain of custody forms; [2] - two replicate injections of the same sample were done

APPENDIX III

REPRESENTATIVE CHROMATOGRAMS

Appendix III-a

Representative Chromatogram of Diphacinone Analytical Standard for the Bait Analysis
(11.8 µg/mL)



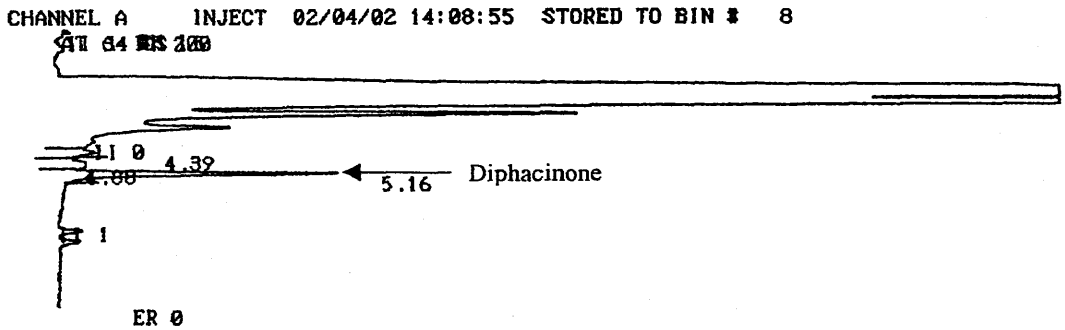
00005-BAIT/VM 02/04/02 12:40:25 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 4 INDEX 4 BIN 4

PEAK#	AREA%	RT	AREA	BC
1	100.	5.18	702567	41
TOTAL	100.		702567	

Appendix III-b

Representative Chromatogram of Ramik Green Bait Sample



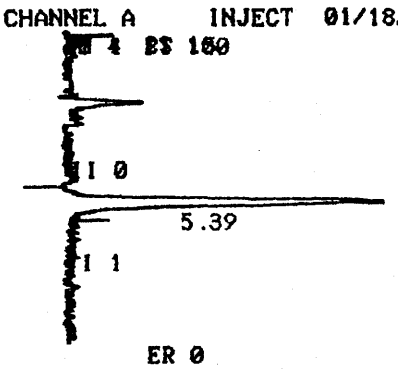
00005-BAIT/VM 02/04/02 14:08:55 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 8 INDEX 8 BIN 8

PEAK#	AREA%	RT	AREA	BC
1	7.3	4.39	27087	41
2	11.275	4.88	41834	42
3	81.425	5.16	302127	43
TOTAL	100.		371048	

Appendix III-c

Representative Chromatogram of Diphacinone Analytical Standard for the Liver Analysis (0.59 µg/mL)



Diphacinone

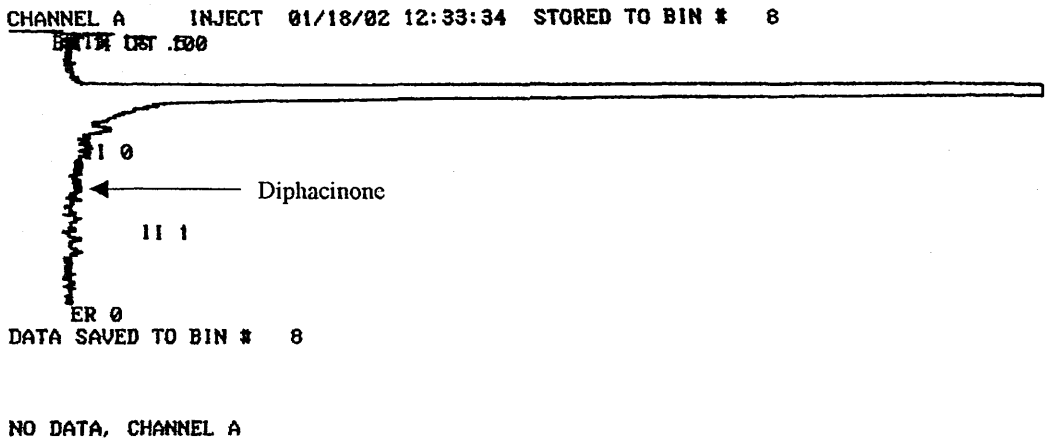
00005-LIVER/VM 01/18/02 11:40:17 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 4 INDEX 4 BIN 4

PEAK#	AREA%	RT	AREA	BC
1	100.	5.39	33626	41
TOTAL	100.		33626	

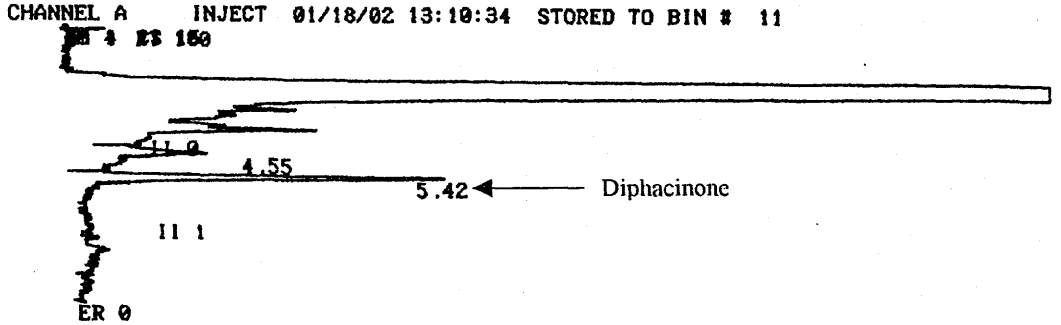
Appendix III-d

Representative Chromatogram of the Liver Where No Diphacinone Was Found



Appendix III-e

Representative Chromatogram of the Liver With a Presence of Diphacinone



00005-LIVER/VM 01/18/02 13:10:34 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 11 INDEX 11 BIN 11

PEAK#	AREA%	RT	AREA BC
1	22.134	4.55	7459 41
2	77.866	5.42	26240 41
TOTAL	100.		33699

Appendix 17. Standard operating procedures.

SOP BRD-04: Live-trapping rats (*Rattus* spp.)
SOP BRD-09: Handling, weighing, and ear-tagging rats (*Rattus* spp.) under field conditions.
SOP BRD-10: Placing radio transmitters on rats (*Rattus* spp.) under field conditions.
SOP BRD-11: Placing non-toxic census blocks in the field to estimate rat (*Rattus* spp.) densities.
SOP BRD-12: Evaluation of rodenticide pellets for degradation and disappearance.
SOP BRD-13: Radio-tracking techniques for marked rats (*Rattus* spp.).
SOP BRD-14. Calibration and use of Pesola scale.
SOP BRD-15: Personal protective equipment for field research studies
SOP BRD-17: Hand-broadcasting rodenticide pellets.

SOP BRD-04: Live-trapping rats (*Rattus* spp.)

1. Purpose

To standardize methods for live-trapping rats and to ensure humane treatment of animals and compliance with the Animal Welfare Act.

2. Traps

Modified wire-cage Japanese live traps (13 cm × 21 cm × 27 cm).

3. Procedure

3.1 At least 3 days before the traps are to be set, pre-bait the area by broadcasting grated coconut (or whatever bait will be used) on the ground in the vicinity where the trap will be set.

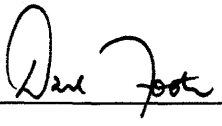
3.2 Select a flat spot on the ground, a branch, or other appropriate site and clear away any brush, vegetation, or debris. Whenever possible, place traps under vegetation or other cover to reduce exposure to the elements and to minimize capture of non-target species. Be sure to leave enough clearance for the trap to close. If practical, put the traps out, unset, a few days ahead of time.

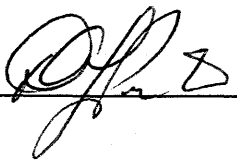
3.3 If trapping on the ground, use a waterproof marker and wire stake flags or colored flagging to identify the trap number, to secure the trap, and to facilitate relocation. If trapping in trees, use wire/rope, or other means to secure the traps to branches, and flagging to identify traps.

3.4 Place a piece of fresh coconut or other bait on the treadle and set the trigger. Cover the trap with a piece of black plastic to protect captured animals from exposure to rain.

3.5 Check traps daily as soon after sunrise as possible. Release any nontarget captures immediately. Rats should be marked (see SOP BRD-09) and released at the site of capture.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director:  Date: 8/25/03

QA Officer:  Date: 8/25/03

SOP BRD-09: Handling, weighing, and ear-tagging rats (*Rattus* spp.) under field conditions.

1. Purpose
   1.1 To standardize methods for handling, weighing, and ear marking live rats in the field and to ensure humane treatment of animals.
   1.2 To ensure the safety of persons handling animals.

2. Procedure
   2.1 Lay out Pesola scale (1 kg) with hook attachment, ear-tag pliers with ear-tags. Record capture site, trap number, rat species, and ear-tag numbers on "Rite-in-the-Rain" record sheet.
   2.2 A leather glove on the hand holding the animal around its neck and long pants are required to prevent exposure to blood/body fluids of animals (see SOP BRD-15).
   2.3 Attach Pesola scale (SOP BRD-14) to trap containing the rat. Record weight of trap and rat.
   2.4 Secure opening of cloth bag (size 13" × 21") around the entrance of the live-trap. Open the live-trap allowing the rat to go into the bag. Place left hand (if you are right-handed) around the top of the bag to prevent the rat from escaping and remove the trap from the cloth bag.
   2.5 With your right hand gently move rat to top of bag, then secure the base of the rat's tail with thumb and forefinger. Open the top of the bag so the rat's head is exposed while maintaining your grip on the base of the tail so the rat cannot escape. Gently slide your left hand (with leather glove on) over the rat's back, placing your thumb and forefinger around the rat's neck. Squeeze firmly so rat cannot escape. Remove rat from the bag.
   2.6 While holding the rat with your left hand, attach an ear-tag to the lower part of the right and left ears. Be sure to slip the ear-tag all the way on the pinna and to clamp it tightly.
   2.7 Examine the rat for sex and reproductive condition.
   2.8 Release the rat at the capture site.
   2.9 Weight the empty trap with the Pesola scale. Record empty trap weight, sex and reproductive condition of the rat.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

SOP BRD-10: Placing radio-transmitters on rats (*Rattus* spp.) under field conditions.

1. Purpose
   1.1 To standardize methods for attaching radio-transmitters on rats under field conditions and to ensure humane treatment of animals.
   1.2 To ensure the safety of persons handling animals.

2. Procedure
   2.1 Lay out black plastic sheet on the ground. Sheet will be used to place the rat on while attaching the transmitter. Check the transmitter frequency with the telemetry receiver and record the transmitter number, frequency, and telemetry receiver coordinates for the transmitter in the rite-in-the-rain record book.
   2.2 Weigh, sex and ear-mark the rat following SOP BRD-09. Place a cotton ball containing Metaphane (see SOP BRD-15) in a gallon-size Ziplock bag. Place rat inside the Ziplock bag until exposure to the Metaphane fumes renders the rat unconscious. Remove the rat from the Ziplock bag and place it on the black plastic sheet. Close the Ziplock bag to prevent fumes from escaping.
   2.3 Secure the transmitter around the neck of the rat. Ensure that the transmitter is attached tight enough so it will not slip off, and loose enough so it will not choke the rat.
   2.4 After the transmitter is attached, lay the rat on the ground under vegetative cover at the capture site. Observe the rat until it recovers and escapes naturally.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

SOP BRD-11: Placing non-toxic census bait blocks in the field to estimate densities of rats (*Rattus* spp.).

1. Purpose
   To standardize methods for placing nontoxic census blocks in the field to estimate densities of rats (*Rattus* spp.).

2. Census blocks
   Nontoxic gnaw blocks.

3. Procedures
   3.1 Select a flat spot on the ground adjacent to a downed log, standing tree, or vegetation. Whenever possible, place the gnaw block under cover to reduce exposure to the elements. Secure the gnaw block to the ground by inserting a 24" or 36" wire stake flag through the hole in the center of the block and into the ground.
   3.2 Use a waterproof marker and wire stake flag to identify the gnaw block (i.e., Plot number, transect number, gnaw block number).
   3.3 In the laboratory, study examples of rat, mouse, and invertebrate (slug) gnawing on blocks until familiar with each type of gnawing.
   3.4 Check each gnaw block daily for two days. Record plot, location, and animal activity (incidents of rat and/or mouse and invertebrate gnawing) in rite-in-the-rain record sheets for each day.

Prepared by: G.D. Lindsey Date: 3 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/23/03

SOP BRD-12: Evaluation of rodenticide pellets for degradation and disappearance.

1. Purpose
To standardize methods for measuring degradation and disappearance of rodenticide pellets under field conditions

2. Baits
Ramik® Green rodenticide pellets (0.005% diphacinone).

3. Procedures
3.1 Randomly select 20 locations within the first quarter of study area to place individual pellets. Mark the pellet location with a wire flag. Place the pellet so it is touching the wired flag to indicate any movement of the pellet. Use a permanent marker to record the location and pellet number on the wire flag.

3.2 Record the condition of each pellet daily on Rite-in-the-Rain record sheet.
a) Date and time and name of recorder.
b) Pellet present or absent (taken by animal).
c) Length and width of pellet measured with a calipers to determine swelling.
d) Softness of each pellet using a metal probe placed at top center of the pellet and pressed down lightly until resistance is met. Pull out the probe and measure the depth the probe using a calipers.
e) Visual percent of surface area of pellet gnawed by rat, mouse, or invertebrate, and cracked because of swelling from moisture.
f) Visual percent of surface area of pellet covered with mold.

3.3 Visual percent of surface area calculated as follows:
a) Top surface of pellet = 25.0%
b) Left side of pellet = 25.0%
c) Right side of pellet = 25.0%
d) Each end of pellet = 12.5%

Prepared by: G.D. Lindsey Date: 3 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

SOP BRD-13: Radio-tracking techniques for marked rats (*Rattus* spp.).

1. Purpose
   To standardize methods for radio-tracking of marked rats.

2. Radio Equipment
   Telonics TR-4 receiver
   RA-14 directional, hand-held, 2-element antenna with flexible element
   Ear phones

3. Procedures
   3.1 Nighttime radio-tracking
   a). Walk into study area using headlamps.
   b). Identify and locate signal of each radio-marked rat using 2-element antenna.
   c). Listen to each signal without moving antenna or receiver for 4 minutes. If signal strength is fluctuating, then record animal as moving. If signal strength is steady, then record animal as not moving.

   3.2 Daytime radio-tracking
   a). Identify and locate signal of radio-tagged rat. Listen to the signal without moving the antenna for 4 minutes. If signal strength is fluctuating, then record animal as moving. If signal strength is steady, then record animal as not moving.
   b). Determine direction of signal by moving antenna in a 180 degree arc. Loudest signal will identify the direction the signal is coming from. Move to another location to obtain a cross-angle (≥30 degrees) from the first signal bearing.
   c). Walk toward the signal until signal strength increases. As you get closer, continue to obtain cross-angle bearings until the rat's location is pinpointed.
   d). Record the rat's location (distance and compass direction from the nearest transect marker). Mark location with colored flagging marked with the rat number, date, and observer's initials.
   e). Record location of rat, i.e., in nest, in tree, on ground, under ground, etc.
   f). If rat is in a nest, mark the location of the nest using colored flagging and determine its location to the nearest transect marker. Record data in daily record sheet.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

SOP BRD-14: Calibration and use of the Pesola scale.

1. Purpose
   To standardize calibration and use of the Pesola scale.

2. Equipment
   Pesola scale

3. Procedures
   3.1 To adjust to zero, turn the knob to left or right until line on scale is aligned to zero.
   3.2 Attach a calibration weight (50-g & 500-g weights) to the scale and record weight. The weight should be within the control limits and weight-range of the Pesola scale.
   3.3 Attach the lower hook of the Pesola scale to the handle of the trap (with the rat inside), hold the scale at the top hook, and read the weight in grams. The scale is marked in increments of 10 grams. Read the weight to the nearest 5 grams.
   3.4 Remove the rat from the trap, then again weigh the trap (without the rat).
   3.5 Substrate the weight of the trap with the rat from the weight of the trap without the rat to determine the weight of the rat.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

SOP BRD-15: Personal Protective Equipment for field research studies

1. 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).

2. Safety concerns

2.1 Handling of rats, mice, feral cats, and mongooses.
2.2 Distribution of Ramik® Green rodenticide pellets (0.005% diphacinone).
2.3 Use of Metaphane to anesthetize rats for attaching radio transmitters.

3. Procedure

3.1 Handling of wild animals may expose personnel to leptospirosis and Hepatitis E.

a). All personnel will read Hawaii Department of Health pamphlet on leptospirosis and publication on Hepatitis E. BRD recommends that employees wear protective clothing (gloves, boots, long pants), and following procedures in SOP BRD-09 are required when handling rats.)

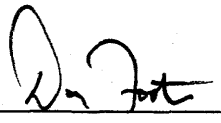
3.2 Ramik® Green.

a). Read MSDS sheet for Ramik® Green.
b). Wear long-sleeved shirt, long pants, boots and rubber gloves under cotton gloves to distribute bait pellets.

3.3 Metaphane

a). Read MSDS sheet for Metaphane.
b). Use in well-ventilated area or out-of-doors.
c). In field, stand upwind when using Metaphane. Saturate cotton ball with Metaphane. Place cotton ball with Metaphane in 1-gallon size Ziplock clear plastic bag. Follow procedures in SOP BRD-10 for placing transmitter on rat.

Prepared by: G.D. Lindsey Date: 14 September 1999

Study Director:  Date: 8/25/03

QA Officer:  Date: 8/25/03

SOP BRD-17: Hand-broadcasting rodenticide pellets.

1. Purpose

To standardize hand-broadcast baiting procedures and to ensure personnel safety standards are followed.

2. Procedure

2.1 Personnel safety for hand-distribution of Ramik® Green rodenticide pellets (0.005% diphacinone).

a). All personnel will wear long-sleeved shirt, long pants, boots, and latex or rubber gloves. Cotton gloves can be worn over the latex gloves to protect latex or rubber gloves.

b). Pellets are placed in plastic bag. The plastic bag containing the pellets is placed in a nylon bag for added protection from spillage.

2.2 Distribution of baits.

a). Employee walks along transect with bag of rodenticide pellets. At established locations the employee reaches into the bag and removes the designated number of pellets. Pellets are individually thrown at designated distances from the transect.

b). Employee walks to the next location and repeats the process until the entire area is baited.

c). Plastic and nylon bags, and gloves, are gathered together and cleaned or disposed of according to label instructions.

Prepared by: G.D. Lindsey Date: 8 October 1999

Study Director: _____ Date: 8/25/03

QA Officer: _____ Date: 8/25/03

Appendix 18. Dates of bait application and monitoring activities.

(a) Wet forest

Bait application	Live-trapping	Census bait blocks	Snap trapping*
7 & 12 Oct 1999	28 Sep – 1 Oct 19 Oct – 22 Oct	5 Oct – 6 Oct 26 Oct – 27 Oct	22 Sep – 23 Sep 28 Oct – 29 Oct
8 & 14 Dec 1999	30 Nov – 3 Dec 4 Jan – 7 Jan	23 Nov – 24 Nov 22 Dec – 23 Dec	18 Nov – 19 Nov 28 Dec – 29 Dec
8 & 14 Feb 2000	1 Feb – 4 Feb 29 Feb – 3 Mar	25 Jan – 26 Jan 23 Feb – 24 Feb	27 Jan – 28 Jan 7 Mar – 8 Mar
12 & 17 Apr 2000	4 Apr – 7 Apr 2 May – 5 May	30 Mar – 31 Mar 25 Apr – 26 Apr	28 Mar – 29 Mar 27 Apr – 28 Apr
14 & 19 Jun 2000	6 Jun – 9 Jun 11 Jul – 14 Jul	31 May – 1 Jun 27 Jun – 28 Jun	2 Jun – 3 Jun 29 Jun – 30 Jun
31 Aug & 5 Sep 2000	22 Aug – 25 Aug 19 Sep – 22 Sep	15 Aug – 16 Aug 12 Sep – 13 Sep	
9 & 15 Nov 2000	31 Oct – 3 Nov 28 Nov – 1 Dec	26 Oct – 27 Oct 21 Nov – 22Nov	
18 & 22 Jan 2001	9 Jan – 12 Jan 6 Feb – 9 Feb	4 Jan – 5 Jan 1 Feb – 2 Feb	
19 & 23 Mar 2001	6 Mar – 9 Mar 10 Apr – 13 Apr	1 Mar – 2 Mar 5 Apr – 6 Apr	
25 & 29 May 2001	15 May – 18 May 19 Jun – 22 Jun	10 May – 11 May 14 Jun – 15 Jun	
23 & 27 Aug 2001	14 Aug – 17 Aug 11 Sep – 14 Sep	9 Aug –10 Aug 6 Sep – 7 Sep	
17 & 19 Dec 2001	11 Dec – 14 Dec 8 Jan – 11 Jan	6 Dec – 7 Dec 3 Jan – 4 Jan	4 Dec – 5 Dec 15 Jan – 16 Jan

*Snap trapping was not done between August 2000 and August 2001.
Mist netting of birds was carried out on 3, 8, 9, 10, 15, 16 November 1999.

(b) Mesic forest

Bait application	Live-trapping	Census bait blocks	Snap trapping
27 Jan & 1 Feb 2000	19 Jan – 22 Jan 8 Feb – 11 Feb	25 Jan – 26 Jan 15 Feb – 16 Feb	6 Jan – 7 Jan 17 Feb – 18 Feb
	21 Mar – 24 Mar	16 Mar – 17 Mar	14 Mar – 15 Mar
	25 Apr – 28 Apr		
	16 May – 19 May	9 May – 10 May	11 May – 12 May
5 & 10 Jul 2000	27 Jun – 30 Jun 25 Jul – 28 Jul	20 Jun – 21 Jun 18 Jul – 19 Jul	22 Jun – 23 Jun 20 Jul – 21 Jul
	29 Aug – 1 Sep	22 Aug – 23 Aug	
25 & 30 October 2000	17 Oct – 20 Oct 14 Nov – 17 Nov	12 Oct – 13 Oct 8 Nov – 9 Nov	
	19 Dec – 22 Dec	15 Dec – 16 Dec	
	30 Jan – 2 Feb	25 Jan – 26 Jan	
	20 Mar – 23 Mar	15 Mar – 16 Mar	
	24 Apr – 27 Apr	17 Apr – 18 Apr	
	5 Jun – 8 Jun	31 May – 1 Jun	
	24 Jul – 27 Jul	19 Jul – 20 Jul	
	3 Sep – 8 Sep	30 Aug – 31 Aug	
	5 Mar – 8 Mar		

Mist netting of birds was carried out on 17, 18, 22, 23, 24, 28 February 2000.
Shooting kalij pheasants was done on 16 February 2000.

Appendix 19. Landcare Research toxicology laboratory analysis reports.

TOXICOLOGY LABORATORY
ANALYSIS REPORT

Manaaki Whenua
Landcare Research

Gerald Street
P.O. Box 69
Lincoln

Ph: +64 3 325 6700
Fax: +64 3 325 2418

Centre for Environmental Toxicology

Report No.: T1764

CLIENT: Eric Spurr, Landcare Research, Lincoln.
CLIENT'S REFERENCE No.: 4 44 0 09 09 01
SAMPLES: 57 of liver
REQUIREMENT: Examine for diphacinone
RECEIVED: 7 March 2002

SAMPLE DESCRIPTION AND IDENTIFICATION:

57 plastic bags were received with samples of liver tissue for analysis. The details were entered into the laboratory sample system, the samples given a reference and stored at -18EC prior to analysis. The samples were received in good condition, except for 4009, 4028 which were too badly decomposed to allow identification and removal of the liver. The sample details and results are as follows:

This report replaces report T1650 of 1.7.02 and includes additional descriptive wording for the client.

Lab No.	Description	Diphacinone, µg/g	MDL
3984	Liver tissue, sample #3, black rat	8.1	
3985	Liver tissue, sample #4, black rat	3.0	
3986	Liver tissue, sample #5, black rat	0.47	
3987	Liver tissue, sample #6, black rat	3.6	
3988	Liver tissue, sample #7, black rat	2.1	0.2
3989	Liver tissue, sample #8, black rat	4.1	
3991	Liver tissue, sample #11, black rat juvenile	<MDL	
3992	Liver tissue, sample #12, black rat	0.55	
3993	Liver tissue, sample #10, Japanese white eye TR16	<MDL	0.3
3994	Liver tissue, sample #13, Japanese white eye TR16	<MDL (0.23)	0.3
3995	Liver tissue, sample #14, Japanese white eye TR16	<MDL	0.4
4000	Liver tissue, sample #18, black rat tr 16	1.9	
4002	Liver tissue, sample #20, black rat	0.21	
4003	Liver tissue, sample #23, black rat	5.0	
4004	Liver tissue, sample #24, black rat	1.8	
4005	Liver tissue, sample #25, black rat	3.0	
4006	Liver tissue, sample #27, black rat	3.6	
4007	Liver tissue, sample #28, black rat	3.7, 3.5	
4008	Liver tissue, sample #1, black rat	<MDL	
4009	Sample #2, black rat	NT	
4010	Liver tissue, sample #3, black rat	6.5	
4011	Liver tissue, sample #4, black rat	2.1, 2.6	
4012	Liver tissue, sample #5, black rat	3.5	
4013	Liver tissue, sample #6, black rat	12	
4016	Liver tissue, sample #7, JAWE	<MDL (0.32)	0.4
4017	Liver tissue, sample #9, Leiothrix	2.9	0.3
4018	Liver tissue, sample #10, Leiothrix	2.8	0.5
4019	Liver tissue, sample #12, Leiothrix	3.9	0.2
4020	Liver tissue, sample #13, JAWE	<MDL	0.4
4021	Liver tissue, sample #14, Leiothrix	4.9	0.3
4022	Liver tissue, sample #17, Leiothrix	1.8	0.5

4028	Sample #25A, N Cardinal (M)	NT	
4029	Liver tissue, sample #33, Nth Cardinal (F)	<MDL	0.3
4030	Liver tissue, sample #34, JAWE	<MDL	0.5
4031	Liver tissue, sample #35, JAWE	<MDL	0.2
4032	Liver tissue, sample #36, Nth Cardinal (M)	<MDL	0.2
4033	Liver tissue, sample #37, Nth Cardinal (F)	<MDL	0.2
4034	Liver tissue, sample #38, Leiothrix	<MDL (0.26)	0.3
4035	Liver tissue, sample #39, Leiothrix	0.28	0.2
4036	Liver tissue, sample #40, Leiothrix	<MDL	0.3
4037	Liver tissue, sample #41, Leiothrix	<MDL	0.4
4038	Liver tissue, sample #38, black rat KI	3.6	
4040	Liver tissue, sample #49, black rat	1.5	
4041	Liver tissue, sample #51, black rat	3.7	
4042	Liver tissue, sample #52, black rat	3.8, 7.1	
4043	Liver tissue, sample #53, black rat	<MDL	
4044	Liver tissue, sample #56, mongoose	1.2, 1.5	
4045	Liver tissue, sample #60, black rat	<MDL	
4046	Liver tissue, sample #61, black rat	4.5, 5.2	
4047	Liver tissue, sample #62, black rat	3.4	
4066	Liver tissue, sample #19, house mouse	2.1	
4067	Liver tissue, sample #20, house mouse	2.4	
4068	Liver tissue, sample #21, house mouse	3.8	
4073	Liver tissue, sample #26, house mouse	0.42	
4076	Liver tissue, sample #29, house mouse	1.3	
4266	Liver tissue, sample #26, lower ki 01/31/02 kalij &	<MDL (0.08)	
4272	Liver tissue, sample #32, lower ki ko 2/13/02 & kalij	<MDL	

NT = not tested.
The results have been adjusted for method recovery. All results are reported to two significant figures.

The determination was carried out using Landcare Toxicology Laboratory Method TLM 048, the determination of diphacinone in liver tissue by HPLC. The method detection limit (MDL) is 0.1µg/g for a 2g sample; variations due to small sample shown above. The uncertainty of the method (95% c.i.) is ± 48%.

TESTED BY: cdr WORKBOOK REF: 19/14, 15, 16, 18, 19 TEST PERIOD: 17/6-1/07/02

AUTHORISED BY:

C.D. Radford, G.R.G. Wright
Approved Signatories
DATE: 11 October 2002

laboratory

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results relate only to the samples as received and tested.. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in the National Vertebrate Pesticide Database.

Column: Luna C8

Mode: Gradient

Operator: cdr
Injection Date:6/19/02 03:39:12 p
Report Date: 6/20/02 00:00:13 p

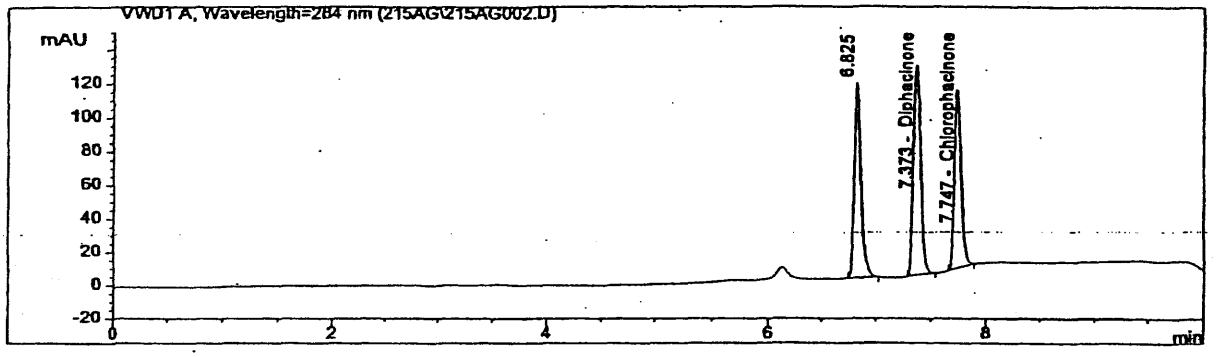
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.0

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

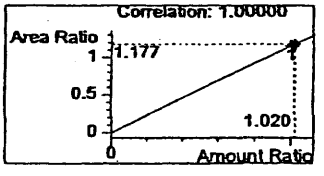
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

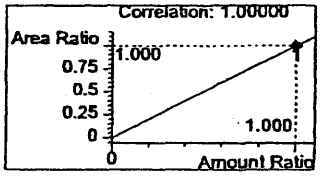
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 00:00:12 pm



Sample: 1.0ug Diph QC

#	Compound Name	Amount	Response F	Response	Height	Area	Meas. R
1	Diphacinone	1.0202	0.8665	540.8987	124.8595	540.8987	7.373
2	Chlorophacinone	1.0000	1.0000	459.4013	105.9892	459.4013	7.747





Column: Luna C8

Mode: Gradient

Operator: cdr
Injection Date:6/19/02 05:14:35 p
Report Date: 6/20/02 11:57:07 a

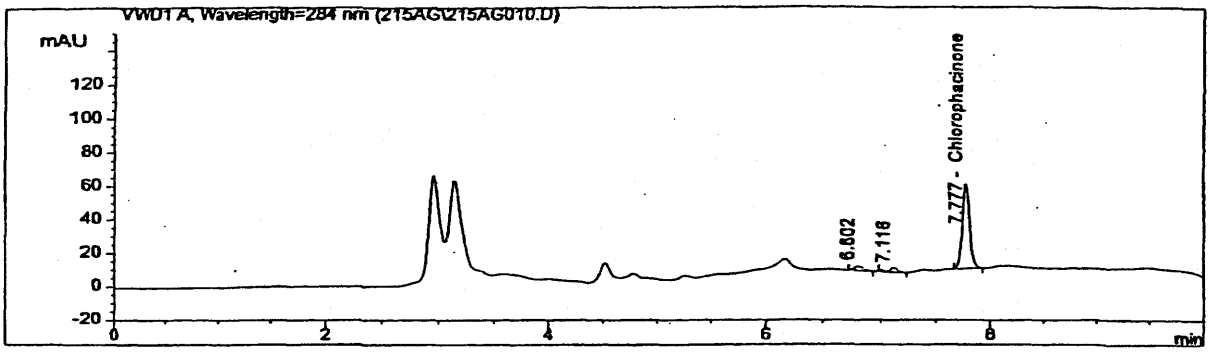
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.6

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

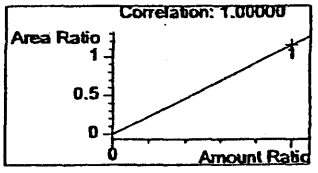
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

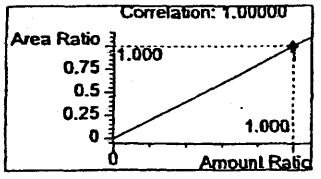
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 11:54:17 am



Sample: 3991

#	Compound Name	Amount	Response F	Response	Height	Area	Meas. R
1	Diphacinone	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
2	Chlorophacinone	0.5000	1.0000	242.1292	50.8972	242.1292	7.777





Column: Luna C8

Mode: Gradient

Operator: cdr
Injection Date:6/19/02 05:26:30 p
Report Date: 6/20/02 11:57:11 a

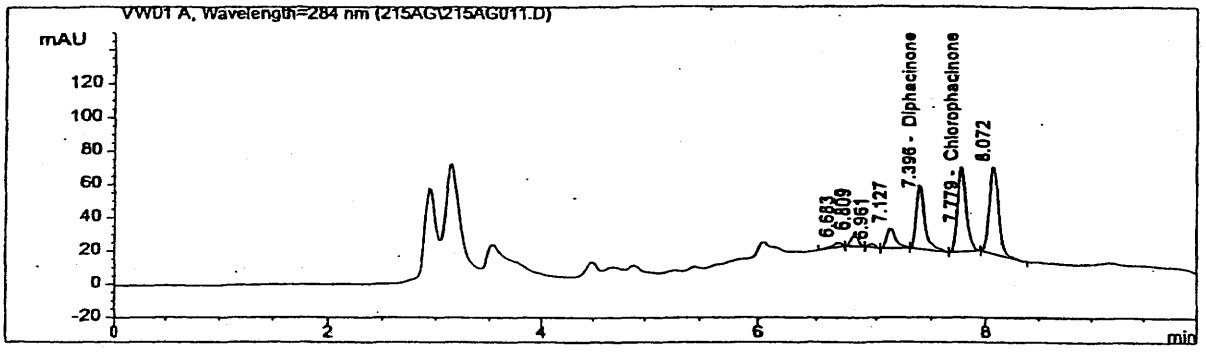
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.5

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

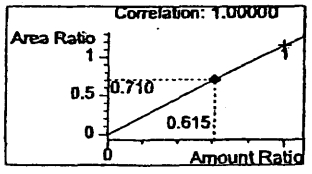
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

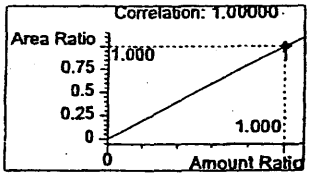
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 11:54:17 am



Sample: 3992

#	Compound Name	Amount	Response F	Response	Height	Area	Meas. R
1	Diphacinone	0.3074	0.8665	198.4172	38.3301	198.4172	7.396
2	Chlorophacinone	0.5000	1.0000	279.6192	51.0195	279.6192	7.779





Toxicology Laboratory Analysis Report

Manaaki Whenua
Landcare Research

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Centre for Environmental Toxicology

Report No: T1794

CLIENT: Eric Spurr, Landcare Research, Private Bag 6, Nelson

CLIENT REFERENCE No.: 444009 0604 **Telephone No:** 03 548 1082

SAMPLES: Six of liver tissue

REQUIREMENT: Examine for diphacinone

RECEIVED: 7 March 2002

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 6

LabNo.	Description	Diphacinone, µg/g
3996	Liver tissue, #34, Japanese white eye	<MDL
3997	Liver tissue, #35, Japanese white eye	<MDL
3998	Liver tissue, #39, Japanese white eye	<MDL
3999	Liver tissue, #50, Nth American cardinal	<MDL
4142	Liver tissue, #8, black rat channel 27, Olaa, 10/14/99	3.6, 3.5
4143	Liver tissue, #9, black rat 2m from 850, Olaa, 10/15/99	3.9

MDL for 3996, 0.5µg/g; 3997, 0.7µg/g; 3998, 0.8µg/g; 3999, 0.3µg/g, due to small size of sample.

The results have been adjusted for method recovery. All results are reported to two significant figures.

The determination was carried out using TLM048, the determination of diphacinone in liver tissue by HPLC. The method detection limit (MDL) is 0.1µg/g and the uncertainty (95% c.i.) is ± 42%.

TESTED BY: cdr **WORKBOOK REF:** 21/18

TEST PERIOD: 13-28/11/02

AUTHORISED BY:

C.D. Radford, G.R.G. Wright
Approved Signatories
Date: 28/11/2002

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in the National Vertebrate Pesticide Residue Database.

Appendix 20. Dates and results of non-target carcass searches.

(a) Wet forest

Bait application date	Search date (d/m/y)	Non-target carcasses found	
		Treatment plot	Non-treatment plot
7 & 12 Oct 1999	24 Sep 99	0	0
	22 Oct 99	0	0
8 & 14 Dec 1999	24 Nov 99	0	0
	29 Dec 99	0	0
8 & 14 Feb 2000	8 Feb 00	0	0
	25 Feb 00	0	0
12 & 17 Apr 2000	29 Mar 00	0	0
	1 May 00	0	0
14 & 19 Jun 2000	8 Jun 00	0	0
	10 Jul 00	0	0
31 Aug & 5 Sep 2000	18 Aug 00	0	0
	13 Sep 00	0	0
9 & 15 Nov 2000	30 Oct 00	0	0
	27 Nov 00	0	0
18 & 22 Jan 2001	5 Jan 01	0	0
19 & 23 Mar 2001	9 Mar 01	0	0
	13 Apr	0	0
25 & 29 May 2001			
23 & 27 Aug 2001			
17 & 19 Dec 2001			

(b) Mesic forest

Bait application date	Search date (d/m/y)	Non-target carcasses found	
		Treatment plot	Non-treatment plot
27 Jan & 1 Feb 2000	14 Jan 00	0	0
	11 Feb 00	0	0
	13 Mar 00	0	0
	10 May 00	0	0
5 & 10 Jul 2000	6 Jul 00	0	0
	21 Jul 00	0	0
	1 Sep 00	0	0
25 & 30 October 2000	16 Oct 00	0	0
	15 Nov 00	0	0
	29 Jan 01	0	0
	27 Apr 01	0	0

Appendix 21. Signs of diphacinone poisoning in radio-collared rats found dead

(a) Wet forest (bait applied 7 and 12 October 1999)

Rat number			Necropsy results			
Tx #	BRD #	LCR #	Internal bleeding	External bleeding	Green dyed gut	Diphacinone (ppm) in liver
41	5	3986	+	+	+	0.47
55	6	3987	+	+		3.6
62	4	3985	+	+	+	3.0
68	3	3984	+		+	8.1
27	8	4142	+	+	+	3.6
Total or average			5/5	4/5	4/5	3.8

Tx # is the radio-transmitter number, BRD # is Biological Resources Division number, LCR # is Landcare Research toxicology laboratory number. See Appendix 19 for details.

(b) Mesic forest (bait applied 27 January and 1 February 2000)

Rat number			Necropsy results			
Tx #	BRD #	LCR #	Internal bleeding	External bleeding	Green dyed gut	Diphacinone (ppm) in liver
87	23	4003	+		+	5.0
89	24	4004	+	+		1.8
79	25	4005	+	+	+	3.0
74	27	4006	+	+	+	3.6
95	38	4038	+			3.6
Total or average			5/5	3/5	3/5	3.4

Tx # is the radio-transmitter number, BRD # is Biological Resources Division number, LCR # is Landcare Research toxicology laboratory number. See Appendix 19 for details.

Appendix 22. Descriptions of rat nests.

Wet forest

Four nests were found in the treatment plot, all in hapuu tree ferns (*Cibotium glaucum*). Three nests were in the crown and one in a hollowed out hapuu. The average height of the tree ferns was 3.3 m and the average diameter was 0.3 m. The nests measured 20 × 15 × 16 cm on average, and were lined with hapuu fronds and akala (*Rubus hawaiiensis*).

The locations of 11 nests were found in the non-treatment plot, but the nests were not recovered. Six nests were in hapuu (average 4.3 m high and 0.4 m diameter), four in ohia (average 22 m high and 0.8 m diameter), and one in olapa (10 m high and 0.3 m diameter). The nests were in the crown of hapuu and high up in ohia and olapa.

Mesic forest

The locations of 14 nests were found in the treatment plot, but the nests were not recovered. Seven nests were in koa (average 22 m high and 1.1 m diameter), six in soapberry (average 23 m high and 0.5 m diameter), and one in ohia (14 m high and 1.6 m diameter). The nests were high up.

The locations of 13 nests were found in the non-treatment plot, but the nests were not recovered. Six nests were in soapberry trees (average 20 m high and 0.6 m diameter), four in koa (average 18 m high and 1.2 m diameter), and three in ohia (average 30 m high and 0.9 m diameter). The nests were high up.

Appendix 23. Ear-tagged rats caught in the treatment plots after bait application.

Wet forest

Polynesian rat, adult, female, ear tags 817/822
first captured in treatment plot 12 July 2000,
recaptured in treatment plot 3 November 2000,
2 months after bait application 31 August 2000
(not captured in August 2000 or September 2000).

Black Rat, adult, female, ear tags 4978/4979
first captured in non-treatment plot 21 October 1999,
recaptured in treatment plot 1 February 2000.

Norway Rat, adult, female, ear tags 868/869
first captured in non-treatment plot 3 May 2000,
recaptured in treatment plot 24 August 2000.

Norway Rat, adult, male, ear tags 981/986
first captured in non-treatment plot 3 October 2000,
recaptured in treatment plot 7 March 2001.

Black Rat, adult, female, ear tags 1598/1597
first captured in non-treatment plot 11 December 2001,
recaptured in treatment plot 9 January 2002.

Mesic forest

No ear-tagged rats were caught in the treatment plot after any bait application.

Appendix 24. Locations where radio-collared rats were found dead.

(a) Wet forest (bait applied 7 and 12 October 1999)

Rat Tx no.	Recovered	Location
41	Carcass	In fallen log
55	Carcass	In fallen log
62	Carcass	On ground, under 100% canopy cover
68	Carcass	On ground, partly covered by vegetation
27	Carcass	On ground, partly covered by vegetation

Rat Tx no. is radio-transmitter number.

(b) Mesic forest (bait applied 27 January and 1 February 2000)

Rat Tx no.	Recovered	Location
29	Carcass*	In fallen log
32	Stationary radio signal only	High up in tree
34	Stationary radio signal only	Underground
37	Stationary radio signal only	Underground
42	Stationary radio signal only	High up in tree
47	Stationary radio signal only	Underground
52	Stationary radio signal only	High up in tree
59	Stationary radio signal only	Underground
64	Stationary radio signal only	High up in tree
69	Stationary radio signal only	Underground
74	Carcass	Underground
78	Stationary radio signal only	High up in tree
79	Carcass	On ground, exposed
81	Stationary radio signal only	High up in tree
87	Carcass	Underground
89	Carcass (euthanased)	In dense vegetation
95	Carcass	On ground, exposed

Rat Tx no. is radio-transmitter number.
* Carcass was mostly eaten.

Appendix 25. Diphacinone residues in birds.

Birds were collected in the treatment plots 3–7 weeks after hand-broadcast application of Ramik® Green bait in the wet forest (Olaa Tr. 16) and mesic forest (Kipuka Ki), Hawaii Volcanoes National Park.

Sample No.	Sample Description	Date Collected	Plot Location	Capture Method	Analysis Lab.	Result (ppm)
29	Kalij pheasant (male)	16 Feb 2000	Kipuka Ki	Shot gun	Genesis	0
30	Kalij pheasant (male)	16 Feb 2000	Kipuka Ki	Shot gun	Genesis	0
31	Kalij pheasant (male)	16 Feb 2000	Kipuka Ki	Shot gun	Genesis	0
32	Kalij pheasant (male)	16 Feb 2000	Kipuka Ki	Shot gun	Genesis	0
33	Kalij pheasant (female)	16 Feb 2000	Kipuka Ki	Shot gun	Genesis	0.09
16	Red-billed Leiothrix	15 Nov 1999	Ola'a Tr. 16	Mist net	Genesis	0
17	Red-billed Leiothrix	15 Nov 1999	Ola'a Tr. 16	Mist net	Genesis	0
37	Red-billed Leiothrix	17 Feb 2000	Kipuka Ki	Snap trap	Genesis	0.66
44	Red-billed Leiothrix	23 Feb 2000	Kipuka Ki	Mist net	Genesis	0.33
47	Red-billed Leiothrix	13 Mar 2000	Kipuka Ki	Snap trap	Genesis	0.7
48	Red-billed Leiothrix	15 Mar 2000	Kipuka Ki	Snap trap	Genesis	0
15	Northern Cardinal	10 Nov 1999	Ola'a Tr. 16	Mist net	Genesis	0
24	Northern Cardinal	16 Jan 2002	Ola'a Tr. 16	Snap trap	Genesis	0.39
41	Northern Cardinal	18 Feb 2000	Kipuka Ki	Mist net	Genesis	0
42	Northern Cardinal	18 Feb 2000	Kipuka Ki	Mist net	Genesis	0
43	Northern Cardinal	22 Feb 2000	Kipuka Ki	Mist net	Genesis	0
46	Northern Cardinal	28 Feb 2000	Kipuka Ki	Mist net	Genesis	0
10	Japanese White-eye	3 Nov 1999	Ola'a Tr. 16	Mist net	Landcare	0
11	Japanese White-eye	3 Nov 1999	Ola'a Tr. 16	Mist net	Genesis	0
12	Japanese White-eye	8 Nov 1999	Ola'a Tr. 16	Mist net	Genesis	0
13	Japanese White-eye	8 Nov 1999	Ola'a Tr. 16	Mist net	Landcare	0
14	Japanese White-eye	8 Nov 1999	Ola'a Tr. 16	Mist net	Landcare	0
34	Japanese White-eye	17 Feb 2000	Kipuka Ki	Mist net	Landcare	0
35	Japanese White-eye	17 Feb 2000	Kipuka Ki	Mist net	Landcare	0
36	Japanese White-eye	17 Feb 2000	Kipuka Ki	Mist net	Genesis	0
39	Japanese White-eye	18 Feb 2000	Kipuka Ki	Mist net	Landcare	0
40	Japanese White-eye	18 Feb 2000	Kipuka Ki	Mist net	Genesis	0

See Appendix 16 and 19 for detailed results of diphacinone analyses.