Experimental Use Permit No. 73815-EUP-1
Final Report

STUDY TITLE

Efficacy of aerial broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests

DATA REQUIREMENTS

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

AUTHORS

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

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

LABORATORY PROJECT ID

QA-02 b

CITATION

Spurr, E.B.; Foote, D.; Forbes Perry, C.; Lindsay, G.D. 2003. Efficacy of aerial broa application of baits containing 0.005% diphacinone in reducing rat populations in Ha forests. Unpublished report QA-02, Pacific Island Ecosystems Research Center, Hawa Volcanoes National Park, HI 96718. 189 p.

Experimental Use Permit No. 73815-EUP-1
Final Report

STUDY TITLE

Efficacy of aerial broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests

DATA REQUIREMENTS

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

AUTHORS

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

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

LABORATORY PROJECT ID

QA-02

CITATION

Spurr, E.B.; Foote, D.; Forbes Perry, C.; Lindsay, G.D. 2003. Efficacy of aerial broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests. Unpublished report QA-02, Pacific Island Ecosystems Research Center, Hawaii Volcanoes National Park, HI 96718. 189 p.

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

David Foote
U.S.G.S. Biological Resources Division
Pacific Island Ecosystems Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

19 February 2004
Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was 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
U.S.G.S. Biological Resources Division
Pacific Island Ecosystems Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

Date

Study Director's Management: _____ 2/19/04

David A. Helweg
U.S.G.S. Biological Resources Division
Pacific Island Ecosystems Research Center
3190 Maile Way, Rm 408
Honolulu, HI 96822

Date

Submitter: _____ 2/19/04

William W. Steiner
U.S.G.S. Biological Resources Division
Pacific Island Ecosystems Research Center
3190 Maile Way, Rm 408
Honolulu, HI 96822

Date

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	7 March 2000
In-field Study	18 October 2001
Analytical Chemistry	16 July 2002
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

U.S.G.S. Biological Resources Division
Pacific Island Ecosystems Research Center
Kilauea Field Station
Hawaii Volcanoes National Park, HI 96718

TABLE OF CONTENTS

I. ABSTRACT

Introduced rats (*Rattus rattus, R. exulans,* and *R. norvegicus*) have been implicated in the decline or extinction of numerous species of plants and animals in Hawaii. This study investigated the efficacy and safety of aerial broadcast application of Ramik® Green pelletized baits containing 0.005% (50 ppm) diphacinone in reducing rat populations in a forested 45.56 ha (112.58 acre) treatment plot compared to a 45.56 ha non-treatment plot in Hawaii Volcanoes National Park. All 21 of the radio-collared rats in the treatment plot died within about 1 week of bait application, whereas none of the 18 radio-collared rats in the non-treatment plot died. There was a 99% drop in both the rat capture rate and percentage of non-toxic census bait blocks gnawed by rats in the treatment plot relative to the non-treatment plot 3 weeks after bait application. The one rat captured in the treatment plot 3 weeks after bait application was not ear-tagged (i.e., it was not a recapture), whereas 44% of the 52 rats captured in the non-treatment plot were ear-tagged. This supports the interpretation that the rat captured in the treatment plot was likely to be a post-treatment immigrant rather than a survivor of the treatment. Most of the toxic bait had disappeared from the forest floor within about 1 month of application. The secondary hazard potential to predatory and scavenging birds from dead rat carcasses was low (because more than 90% of rats died in inaccessible locations). No birds were found dead 1 month or 3 months after bait application. The remains of a Hawaiian Hawk (Io) were found 6 months after bait application, but it was not possible to determine the cause of death. We recommend that a case be made to the EPA for registration of aerial-broadcast application of Ramik® Green bait for the control of rat populations in conservation areas in Hawaii.

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 wet forests harbor much of Hawaii's 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 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 (*Herpestes 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).

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

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, rats prey on native Hawaiian tree snails (Hatfield 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 cause them to be the greatest rodent threat to native forest birds. Both Black and Polynesian Rats are also known predators to native ground and burrow nesting birds (Baldwin 1945; Johnson 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).

Fruits and seeds of many endemic plant species are 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 (IUCN 2002; U.S. Fish and Wildlife Service 1996, 2002). In a study carried out in wet montane forests of Maui Island, 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 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).

There are only two methods (trapping and bait stations) 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 predators in small or limited areas. 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 on offshore islands, forests, and other non-crop areas. The fish-flavored products are also registered for use against mongooses. However, trapping and use of bait stations are labor intensive and impractical for controlling predators over large conservation areas. Studies at the

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). Many of the areas where the flora and fauna are threatened by rats are remote and rugged, with limited access. The only cost-effective method for rat control in these areas is the broadcast application of rodenticide bait (Moors et al. 1992; Tobin 1994).

Three preliminary studies have been done in preparation for the development of broadcast baiting for rat control in Hawaii. The first, a standard laboratory feeding bioassay with commercial Ramik® Green pelletized bait, containing 0.005% (50 ppm) diphacinone, determined the minimum exposure times and amounts of bait required to achieve Environmental Protection Agency (EPA)-acceptable control of wild-caught rats from Hawaiian ecosystems. These were 7 days and 37.5 g (15 commercial size pellets) for *R. rattus*, and 6 days and 30.0 g (12 commercial size pellets) for *R. exulans* (Swift 1998). The second, a hand-broadcast bait application rates field study (simulating aerial distribution) using Ramik® Green placebo baits (formulated without diphacinone) and coated with tetracycline hydrochloride (a biological tracer), indicated that an application rate of 22.5 kg/ha would maximize bait exposure to rats, while minimizing excess bait usage (Dunlevy et al. 2000). The third, a hand-broadcast toxic baiting field study (simulating aerial distribution) using Ramik® Green containing 0.005% diphacinone, obtained 100% control of the populations of all three rat species in two 4-ha study areas, one in wet forest and the other in mesic forest (Lindsey and Forbes 2000). Repeated hand-broadcast toxic bait applications in the same study areas were also highly effective (Spurr et al. 2003).

In October 2001, we investigated the effectiveness against rats and safety to non-target species of aerial-broadcast application of 0.005% (50 ppm) diphacinone bait in a Hawaiian mesic forest (Kipuka Ki, Hawaii Volcanoes National Park). The research was conducted under Experimental Use Permit No. 73815-EUP-1 from the EPA (Appendix 1), with the approval of the U. S. National Park Service (NPS) (Appendix 2), U. S. Fish and Wildlife Service (FWS) (Appendix 3), Hawaii Division of Forestry and Wildlife (DOFAW) (Appendix 4), and The Nature Conservancy (TNC) (Appendix 5), and in coordination with the Hawaii Department of Agriculture. An earlier Biological Opinion prepared by the FWS in accordance with Section 7 of the U. S. Endangered Species Act concluded that hand-broadcast application of diphacinone bait in 4-ha forest plots was unlikely to jeopardize the continued existence of the Hawaiian Hawk or Io (*Buteo solitarius*) (Appendix 6). An amendment to that Biological Opinion approved the aerial-broadcast application of diphacinone bait in a 45-ha plot (Appendix 7). An environmental assessment of the proposed study (Appendix 8) was accepted by the NPS (Appendix 9).

B. OBJECTIVES

To develop a safe, economical method to control introduced rats in non-crop conservation areas of Hawaii, by determining:

- The efficacy of aerial-broadcast baiting with 0.005% diphacinone pellet bait in reducing rat populations.
- The disappearance rate of aerially broadcast 0.005% diphacinone pellet bait from the forest floor.
- The secondary hazard potential from aerial-broadcast baiting with 0.005% diphacinone pellet bait.

C. STUDY DESIGN

The study design consisted of a treatment plot and a non-treatment plot, in which rat populations were monitored before and after treatment.

D. STUDY AREAS

The treatment and non-treatment plots measured 675 m × 675 m (45.56 hectares or 112.58 acres), and were located in mesic forest in Hawaii Volcanoes National Park (HAVO) (see Appendix 10 and Appendix 11, Amendment No. 1). The treatment plot was in Kipuka Ki, and the non-treatment plot in Kipuka Puaulu, 1.5 km away. Both kipukas were on ancient areas of deep ash soil surrounded by lava of the late prehistoric Keamoku flows, on the lower slope of Mauna Loa at 1,200–1,360 m elevation.

Vegetation within the central part of the kipukas was composed of a tall Koa/Ohia/Soapberry (*Acacia koa / Metrosideros polymorpha / Sapindus saponaria*) forest. Native ferns and herbs dominated the ground cover where the forest canopy was dense, but Blackberry (*Rubus argutus*) and alien grasses, such as Meadow Ricegrass (*Ehrharta stipoides*) and *Paspalum* spp., were common in some areas. Kipuka Ki also contained some Jerusalem Cherry (*Solanum pseudocapsicum*). Patches of open grassland with scattered trees also occurred 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. Neither area contained surface water impoundments or streams. No buildings or other manmade structures were located within 1 km of the treatment plot.

Fauna within the study plots included potentially eight endemic bird species – the Hawaiian Hawk or Io, Hawaiian Short-eared Owl or Pueo (*Asio flammeus sandwichensis*), Omao, Elepaio (*Chasiempsis sandwichensis*), Apapane (*Himatione sanguinea*), Iiwi (*Vestiaria coccinea*), and Common Amakihi (*Hemignathus virens*), and one endemic mammal – the Hawaiian Hoary Bat (*Lasiurus cinereus semotus*). Introduced bird species included the Barn Owl (*Tyto alba*), Kalij Pheasant (*Lophura leucomelana*), Northern Cardinal (*Cardinalis cardinalis*), Red-billed

Leiothrix (*Leiothrix lutea*), Melodious Laughing Thrush (*Garrulax canorus*), Japanese White-eye (*Zosterops japonicus)*, and House Finch (*Carpodacus mexicanus*) (Baldwin 1941; Stone and Pratt 2002). Introduced mammals included the four rodent species (*Rattus rattus, R. exulans, R. norvegicus*, and *Mus musculus*), the Feral Cat, and Indian Mongoose (Stone and Pratt 2002).

A monitoring grid measuring 275 m × 275 m (7.56 ha) was established in the center of each study plot. The grid consisted of 12 transects 275 m long and 25 m apart. Each transect was flagged with markers at 12.5-m intervals. Four of the transect lines extended 100 m beyond each end of the grid (for a total length of 475 m), and were used for searching for non-target mortality (see below).

III. MATERIALS AND METHODS

The methods and materials were specified in the Experimental Use Permit application (Appendix 10), with amendments (Appendix 11) and deviations (Appendix 12).

A. BAITS

The test bait was a fish-flavored, green-colored, pellet formulation of Ramik® Green (Lot No. 144548, HACCO, Inc., Madison, WI), nominally weighing 6 g and containing 0.005% (or 50 ppm) diphacinone (Appendix 13, Appendix 14). The bait was manufactured on 16 September 2000 (Appendix 15) and received on 16 April 2001 (Appendix 16), so was 7 months old when received.

For quality assurance, the weight of a random sample of 60 individual baits was measured on 19 April 2001. The diphacinone content of a random sample of baits was determined by HACCO, using high performance liquid chromatography (HPLC), at the time of manufacture in September 2000 (Mary Ann Douglas, HACCO, pers. comm., Appendix 15). It was determined again in May 2001 (when the bait was 8 months old) and in February 2002 (when 17 months old) by Genesis Laboratories Inc. (Wellington, CO), also using HPLC (Malkov and Mach 2002, Appendix 17). It was also assessed in May 2002 (when the bait was 20 months old) by HACCO (Mary Ann Douglas, HACCO, pers. comm., Appendix 15).

B. BAIT APPLICATION

The planned total bait application rate was 22.5 kg/ha (20 lb/acre), although the permit allowed up to 28 kg/ha (25 lb/acre). Thus, 1025 kg (2252 lb) of bait was required to cover the 45.56 ha (112.58 acre) treatment plot at the planned application rate. To ensure that the baits were available to rats over the recommended time period of 10–15 days (Dunlevy et al. 2000), one-half of the bait (512.5 kg or 1126 lb) was to be applied at 11.25 kg/ha (10 lb/acre) on day 1 and the other half 5–7 days later. In the event, the first application of bait was made on 25 October and the second on 30 October 2001.

The bait was applied from a specially designed helicopter bait bucket, imported from Lakeland Helicopters, Rotorua, New Zealand (Spurr 2002) (Fig. 1), slung under a Hughes 500D helicopter (Volcano Heli-Tours, Hilo, HI) (see Appendix 11, Amendment No.11). The bucket consisted of a conical hopper with a 150 mm diameter opening in the bottom (that could be closed with a gate) through which bait flowed onto a spinner that spread the bait over the treatment plot. The size of the opening could be varied by inserting rings with different-sized internal diameters into a socket in the bottom of the bucket. The size of the opening in the bottom of the bucket was the sole determinant of the rate of bait application because the helicopter flew at a constant height (approximately 150 feet or 45 m) and constant speed (approximately 60 knots, equivalent to 111 km/h or 30.833 m/s).

The correct size of opening in the bottom of the bait bucket that was needed to achieve a rate of application of 11.25 kg/ha of Ramik® Green baits had to be determined by a flow rate test (Appendix 18). As a result of this test, a wooden ring with an internal diameter of 128 mm was used in the first bait application on 25 October, and a plastic ring with 124 mm internal diameter in the second bait application on 30 October 2001.

The number of helicopter flights required to cover the treatment area was determined by measuring the swathe width (the width that the bait bucket was able to spread baits) in a trial aerial application of placebo Ramik® Green baits on 20 October 2001 (Appendix 19). The size distribution of the placebo bait before and after application was also measured to determine whether there was any significant breakage as a result of being applied from the helicopter bait bucket (Appendix 20). As a result of these preliminary trials, seven helicopter flights were made over the 675-m-wide treatment area, with an average swathe width of 96.4 m (675 m wide / 7 flights = 96.4 m), for each toxic bait application. The helicopter flight path on one return flight (two swathes over the treatment plot) on 30 October 2001 was recorded on a Garmin GPS (global positioning system).

The accuracy of the toxic bait application was assessed by (a) recording the total amount of bait applied to the treatment plot, (b) recording the location of baits on transects through the treatment plot immediately after bait application, and (c) searching for baits at the ends of the swathes to determine if there had been any bait applied outside the boundaries (Appendix 21).

C. BAIT DISAPPEARANCE

The disappearance of baits from the forest floor was carried out according to standard operating procedure (SOP) BRD-12 (Appendix 22). Twenty locations (at least 25 m apart) were randomly selected along transects in the first quarter of the central monitoring area, and marked with a colored wire flag. A bait was then placed beside each marker. The presence or absence of each bait and signs of consumption by rats, and feeding by slugs, snails, and other invertebrates, were recorded daily for 14 days or until the bait disappeared or disintegrated. This is a slight amendment to the protocol outlined in the EUP application (Appendix 11, Amendment No. 5).

D. RAT ACTIVITY INDICES

Rat activity was monitored using three techniques: radio telemetry, live trapping, and non-toxic census bait blocks.

Radio-telemetry. Radio-telemetry was carried out according to SOP BRD-10 and BRD-13 (Appendix 22). Radio-transmitters (Holohil PD-2C, weighing 4.2 g) were fitted to 25 Black rats in the treatment plot (Kipuka Ki) and to 23 Black rats in the non-treatment plot (Kipuka Puaulu), 1 week before the first bait application on 25 October 2001. Four rats in the treatment plot and five rats in the non-treatment plot lost their transmitters before bait application, reducing the sample size to a total of 21 rats in the treatment plot and 18 rats in the non-treatment plot.

Radio-signals from the radio-collared rats were monitored using portable receivers (Telonics TR-4) and hand-held two-element directional antennas (Telonics RA-14) for each of 3 consecutive nights immediately before bait application, to confirm that the rats were alive and active. Following bait application, radio-signals from the radio-collared rats were monitored nightly for 8 nights, until all rats in the treatment plot were dead (see Appendix 12, Deviation No. 1). A fluctuating, variable-strength radio signal indicated that a rat was alive and active, whereas a steady radio-signal indicated that the rat was not moving. Each rat not moving during a nightly monitoring session was tracked down the next morning to determine its location and fate.

Rats recovered dead were necropsied and examined for green bait within 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 analysis of diphacinone residues in the liver.

The effectiveness of bait application was determined statistically using a 2 × 2 Chi-square analysis of the number of radio-collared rats alive vs. dead in the treatment and non-treatment plots before and after bait application.

Live-trapping. Live-trapping was carried out according to SOP BRD-04 and SOP BRD-09 (Appendix 22). A total of 144 Haguruma® wire-cage traps (basket traps) were placed at 25-m intervals on transect lines spaced 25 m apart within each study plot. The traps were left closed for at least 2 weeks before the first trapping to allow the rats time to become accustomed to the traps. The traps remained at the trap locations throughout the study period. Two weeks before bait application in the treatment plot, trap locations were pre-baited with shredded coconut for 3 nights, and then the traps were opened and baited with coconut chunks for 4 consecutive nights (maximum 576 trap-nights). The traps were checked daily, and when possible all rats captured were identified to species, sex, and age class (juvenile or adult), and then weighed, ear-tagged, and released (but see Appendix 12, Deviation No. 2). Traps were opened again in each study plot 3 weeks, 3 months, and 6 months after bait application to determine efficacy and rat reinvasion rates (Appendix 11, Amendment No. 2). As before, the traps were pre-baited with shredded coconut 3 days before they were opened.

Rat capture rates (the number of rats caught 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) (see also Beauvais and Buskirk 1999). That is, 0.5 of a trap-night was deducted from the total number of trap-nights for each target and non-target capture and for each trap sprung without capture.

The percentage reduction in rat capture rates (% kill) from pre- to post-treatment in the treatment plot, relative to the non-treatment plot, was calculated from the formula:

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

where

expected capture rate = capture rate in the treatment plot pre-treatment × (capture rate in the non-treatment plot post-treatment / capture rate in non-treatment plot pre-treatment)

observed capture rate = capture rate in treatment plot post-treatment.

It was not possible to statistically compare rat capture rates per 100 corrected trap-nights in the treatment and non-treatment plots before and after bait application, to assess the effectiveness of bait application, because there was no replication of plots (only pseudo-replication of traps). Chi-square analysis could not be used on the raw data (number of rats caught) because the analysis could not take into account the different number of trap-nights in each trapping session, resulting from different numbers of rats and non-target species (house mice, mongooses, and birds) that were caught, nor could it allow for spatial or temporal correlation between trappings (Appendix 11, Amendment No. 2).

Non-toxic census bait blocks. Non-toxic census bait blocks were monitored for signs of rat gnawing according to SOP BRD-11 (Appendix 22). A total of 132 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 in each study plot, but half-way between the live-trap locations (i.e., at 12.5 m, 37.5 m, 62.5 m, etc. along the transect lines). The census bait blocks were placed on the transect lines 2 weeks before and 1 month, 3 months, and 6 months after bait application to determine efficacy and rat reinvasion rates (Appendix 11, Amendment No. 3). They were attached to the ground using a 1-m wire flag inserted through a hole in the center of the blocks. For each monitoring session, the bait blocks were examined daily for 2 consecutive days (maximum 264 bait nights) for signs of feeding by rats or other animals (viz., house mice, mongooses, birds, and invertebrates) (Appendix 11, Amendment No. 3). Unfortunately, some of the bait blocks went missing between checks. It was not possible to determine which animal species was removing the bait blocks, so percentage interference was calculated on those blocks remaining.

The percentage reduction in the number of census bait blocks gnawed by rats in the treatment plot relative to the non-treatment plot (% kill), 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)

observed number = number in treatment plot post-treatment.

It was not possible to statistically compare rat gnawing on census bait blocks in the treatment and non-treatment plots before and after bait application for the same reasons as for live trapping (viz., because there was no replication of plots) (Appendix 11, Amendment No. 3).

E. MOUSE ACTIVITY INDICES

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

Kill trapping. Fifty-six mouse traps (Victor snap traps) were located at 10 m intervals (two traps per location) along one transect line in each study plot to estimate mouse densities pre- and post-treatment (Appendix 11, Amendment No. 4). Trapping was carried out 3 weeks before, and then 1 month, 3 months, and 6 months after bait application. Within each trapping session, the traps were baited with coconut chunks, set, and examined daily for 2 days (maximum 112 trap nights). Captured mice were placed in marked containers and frozen. Frozen specimens were sent to Genesis Laboratories (Wellington, CO) or Landcare Research (Lincoln, New Zealand) for analysis of diphacinone residues in the liver. The traps were removed after a trapping session, and re-located to a new transect for the next trapping session. Mouse capture-rates pre- and post-treatment in the treatment and non-treatment plots were calculated following the method of Nelson and Clark (1973). That is, 0.5 of a trap night was deducted from the total number of trap nights for each target and non-target capture and for each trap sprung without capture.

Live trapping. House 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. House 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 (hawks and owls) was recorded throughout the study whenever they were observed in the study areas.

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

Four randomly selected transects (475 m long by 5 m wide), spaced at least 25 m apart, in the treatment and non-treatment plots (representing 2% of each plot), were walked to search for non-target mortality on 15 October (2 weeks before bait application), and 13 November, 11 February, and 13 May (3 weeks, 3 months, and 6 months after bait application) (see Appendix 11, Amendment No. 7). Each transect extended 100 m beyond each end of the central study plot. The searching took about 3 person-hours on each sampling occasion. In addition, at least 500 person-hours were spent in each plot carrying out other activities (such as live-trapping, radio-telemetry, and census bait block monitoring) on each sampling occasion. Animals found dead were recorded as to species, weighed, sexed, placed in individually marked containers, frozen, and retained for diphacinone residue analysis.

Birds of four introduced species, viz., Kalij Pheasant, Red-billed Leiothrix, Northern Cardinal, and Japanese White-eye, were collected by shooting or mist-netting in the treatment plot 1 month after the first bait application. Birds (viz., Red-billed Leiothrix) caught in kill-traps set for mice were also collected to increase sample sizes. The birds were frozen, and initial specimens were sent to Genesis Laboratories (Wellington, CO) and later specimens to Landcare Research (Lincoln, New Zealand) for determination of diphacinone residues in the liver (Appendix 11, Amendment No. 6).

Invertebrates found on and/or immediately underneath Ramik® Green baits were recorded at 1–4-day intervals from 26 October to 16 November 2001. Baits were placed at 2.5-m intervals beside wire flags on four transect lines, of 25 baits per line, on 25 October and on another four lines on 30 October 2001. Observations were made during the day, between 0930 and 1300 hours, and at night, using a headlamp, between 1800 and 2000 hours (after sunset), and between 0400 and 0600 hours (before sunrise). Invertebrates were identified to species where possible, otherwise genus, family, order, or class.

G. ENVIRONMENTAL CONDITIONS

A rain gauge and minimum/maximum thermometer were placed in the treatment plot to monitor daily weather conditions. Daily rainfall and temperature were recorded by 10 a.m. each morning for 2 weeks after bait distribution (but see Appendix 12, Deviation No. 5).

IV. RESULTS

A. BAITS

The mean weight of 60 randomly selected baits was 6.29 g (± 0.06 g standard error) per bait. The diphacinone content of a random sample of baits was 51 ppm at the time of manufacture in September 2000 (Table 1). This is within the 40 CFR Part 158.175 certified limits (45–55 ppm). It was still within the certified limits 20 months later (Table 1).

B. BAIT APPLICATION

A total of 635 kg (1400 lb) of bait was applied on 25 October, and 481 kg (1061 lb) on 30 October, for a grand total of 1116 kg (2461 lb) (Appendix 16). This is more than the planned 1025 kg (2252 lb), but still below the permitted level of 1361 kg (3000 lb). The reason for the greater amount of bait being applied on 25 October was because some bait was applied outside the treatment plot, though still within Kipuka Ki (see Appendix 12, Deviation No. 3 for details). The rate of application within the treatment plot was estimated as 11.83 kg/ha (10.51 lb/acre) on 25 October, and 10.56 kg/ha (9.39 lb/acre) on 30 October, for a total of 22.39 kg/ha (19.90 lb/acre).

Searches of 12 transects through the monitoring area in the treatment plot after bait application on 25 October resulted in the finding of 858 baits/ha, or 45.8% of the expected 1875 baits/ha (see Appendix 21). Baits were found on 45.4% of the 1320 plots searched (each 2.5 × 3 m). The largest strip without baits was 42.5 × 3 m. Searches after the second bait application on 30 October, resulted in the finding of 695 baits/ha, or 37.1% of the expected 1875 baits/ha. Baits were found on 37.7% of the plots searched. In addition, 87% of the baits from the first bait application were found, equivalent to 746 baits/ha. Baits from the first application were easier to find than baits from the second application because they had swollen (from absorption of moisture), and were therefore larger, and they had changed to a brighter green color, which contrasted more with the background vegetation. The largest strip without baits after the second application was 82.5 × 3 m.

The helicopter flight path for the first return flight (two swathes over the treatment plot) on 30 October 2001 was close to the prescribed flight path (Fig. 2). GPS recordings of other flight paths were not recorded. From ground searches made at the end of the flight paths, baits were found up to 30 m outside the treatment plot on 25 October, and up to 80 m outside the treatment plot on 30 October (see Appendix 21). This illustrates the difficulty that the pilot faces in deciding when to close the gate in the bottom of the bait bucket.

C. BAIT DISAPPEARANCE

The disappearance rate of baits was more rapid after the first application on 25 October than after the second application on 30 October 2001 (Fig. 3). Rat numbers would have been lower after the second application. However, a similar percentage of monitored baits (25%) remained 2 weeks after each application. From incidental observations, most baits had disappeared 1 month after application. However, the remains of at least one bait were still present on the forest floor on 25 January 2002, 3 months after application. Some remaining baits (number not quantified) had signs of feeding by slugs (smooth, slimy hollows on the surface) and other invertebrates, probably beetles (small particles of bait around the main piece of the remaining bait).

D. RAT ACTIVITY INDICES

Radio-telemetry. All 21 radio-collared Black Rats in the treatment plot died within 9 days of the initial bait application on 25 October 2001 (Table 2). None of the 18 radio-collared Black Rats in the non-treatment plot died in that time. Post-treatment survival of the radio-collared rats in the treatment plot (0 of 21 rats) and non-treatment plot (18 of 18 rats) was significantly different ($\chi^2 = 39.0$, df = 1, $P < 0.001$).

Carcasses of only six of the 21 radio-collared rats in the treatment plot were recovered. Seven of the remainder were inaccessible, either underground in aa rock or high up in old trees, and eight were presumed to have been scavenged, perhaps by mongooses, because the collars were found without a carcass. In addition to the carcasses of the six radio-collared Black Rats, two other Black Rats, including an ear-tagged one, were found dead after bait application in the treatment plot. Necropsies of the eight rats revealed hemorrhaging typical of diphacinone poisoning (Table 3). All had internal hemorrhaging (under skin, around heart, and in lungs, liver, bladder, genitals, thoracic cavity, and abdominal cavity) and five also had external hemorrhaging (from mouth, nose, ear, anus, and genital region). Five of the rats also had green bait in their stomachs and/or green fecal pellets in their intestines. An average of 4.4 ppm diphacinone was detected in the livers (n = 7, because one was too decomposed to analyse) (Table 3, Appendix 24).

Live-trapping. There was a 99% drop in the rat capture rate in the treatment plot relative to the non-treatment plot 3 weeks after bait application (Table 4). One week before bait application, 82 Black Rats, 1 Polynesian Rat, and 1 Norway Rat were captured in the treatment plot, and 74 Black Rats in the non-treatment plot. Three weeks after bait application, however, just 1 Black rat was caught in the treatment plot whereas 50 Black Rats and 2 Polynesian Rats were caught in the non-treatment plot. Three months after bait application, 14 Black Rats were caught in the treatment plot and 22 Black Rats in the non-treatment plot. Six months after bait application, 11 Black Rats and 1 Polynesian Rat were caught in the treatment plot, and 15 Black Rats and 1 Polynesian Rat in the non-treatment plot. Thus, most rats caught were Black Rats. The rat capture rate in the treatment plot was still reduced by 36% relative to the non-treatment plot 6 months after bait application (Table 4).

The one rat captured in the treatment plot in November, 3 weeks after the initial bait application in October, was not ear-tagged (i.e., it was not a recapture from before bait application). Likewise, of the 14 rats captured in February, 3 months after bait application, and 12 rats captured in April, 6 months after bait application, none had been captured and ear-tagged before bait application. In contrast, in the non-treatment plot, 44% of the 52 rats captured in November, 55% of the 22 rats captured in February, and 44% of the 16 rats captured in April had been captured and ear-tagged in October (Table 5).

Proportionately more males and proportionately more juveniles were caught in the treatment plot than in the non-treatment plot after bait application (Table 6). However, most of the rats caught in the treatment plot in the first 6 months after bait application were adult males. This may reflect the time of year (autumn) that the trial poisoning operation was carried out. The rat capture rate in the non-treatment plot declined naturally from October 2001 to May 2002, presumably as a result of no or little breeding or recruitment over winter. Thus, there may have been fewer juveniles available to re-colonize the treatment plot than if the trial poisoning operation had been done in spring or summer.

Non-toxic census bait blocks. Rat gnawing on remaining census bait blocks was reduced by 99% in the treatment plot relative to the non-treatment plot 3 weeks after bait application, and was still reduced in the treatment plot 6 months after bait application (Table 7). In addition, the incidence of missing census bait blocks was reduced by 97% in the treatment plot relative to the non-treatment plot 3 weeks after bait application, and also was still reduced in the treatment plot 6 months after bait application.

E. MOUSE ACTIVITY INDICES

Kill-trapping. The number of House Mice caught in snap-traps was reduced by 79% in the treatment plot relative to the non-treatment plot 3 weeks after bait application (Table 8). The one mouse captured in the treatment plot 3 weeks after bait application contained 3.8 ppm diphacinone in its liver (mouse # 4068 in Appendix 24). Mouse numbers recovered to pre-poison levels 3 months after bait application, and were nearly 3 times pre-poison levels 6 months after bait application.

Live-trapping. The number of House Mice caught in live traps was reduced by 76% in the treatment plot relative to the non-treatment plot 3 weeks after bait application (Table 9). Two of the mice captured in the treatment plot 3 weeks after bait application contained 0.42 and 1.3 ppm diphacinone in their livers (mouse # 4073 and 4076 in Appendix 24). Mouse numbers recovered to near pre-poison levels 3 months after bait application.

Non-toxic census bait blocks. House Mouse gnawing on census bait blocks was reduced by 93% in the treatment plot relative to the non-treatment plot 1 month after bait application (Table 10). Mouse gnawing on bait blocks increased to two-thirds of the pre-poison levels 3 months after bait application.

F. NON-TARGET SPECIES ASSESSMENT

Presence of avian predators. A pair of Hawaiian Hawks was present in the treatment plot throughout the study. They had a juvenile with them at the time of bait application in October 2001. The fate of the juvenile is not known, but the pair was still present 1 month after bait application. The remains of a dead Hawaiian Hawk (feathers, beak, and legs) were found in the treatment plot on 9 May 2002, 6 months after bait application (see below), and the species determination was confirmed by Dr. Storrs Olson of the Smithsonian Institution. One Hawaiian Hawk was seen occasionally in the non-treatment plot throughout the study. No Hawaiian Short-eared Owls were seen in either the treatment or non-treatment plots.

Locations of dead rats. All six of the radio-collared rat carcasses that were recovered after bait application were in locations inaccessible to Hawaiian Hawks or other avian predators (Table 11). One other radio-collared rat (#70), found 4 days after the first bait application, barely alive, bleeding from the ventrum and stumbling along on a fallen branch during the daytime, was exposed to avian predators. Its carcass was not recovered but its radio-collar was found later on the ground surface. Seven other radio-collars were also found without rats, all in locations inaccessible to avian predators. Seven dead radio-collared rats were unable to be recovered, and these were also all in locations inaccessible to avian predators (viz., deep in hollows of old trees or under immovable aa rock). Thus, of the 21 radio-collared rats, 20 (95%) were considered to have died in locations inaccessible to avian predators, and only one (5%) in a location accessible to avian predators.

In addition to the above, one unmarked Black Rat was found barely alive 4 days after the first bait application, stumbling along on the ground surface during the daytime, accessible to avian predators. Also, the carcass of one ear-tagged (but not radio-collared) Black Rat was found dead 7 days after bait application, in underbrush at the base of a tree, inaccessible to avian predators.

Searches for dead non-target species. No dead non-target species were found during any of the ground searches in the treatment or non-treatment plots (in about 3 person-hours, covering 2% of each plot). However, two dead rats (see above), three dead mice, and the remains of a dead Hawaiian Hawk (feathers, beak, and legs) were found during extensive visits to the treatment plot for radio-telemetry, live-trapping, and census bait block surveys (at least 500 person-hours in October before bait application, and again in November, February, and May, 1 month, 3 months, and 6 months after bait application). Two dead mice were found 6 and 7 days after the first bait application, on the ground surface, exposed to avian predators. They contained 2.1 and 2.4 ppm diphacinone in their livers (mouse # 4066 and 4067 in Appendix 24). The third dead mouse was found 9 days after the first bait application, in a nest under a log inaccessible to avian predators. The diphacinone concentration in its liver was not determined. The remains of the Hawaiian Hawk were found on 9 May 2002, 6 months after bait application. There was no evidence to indicate whether the bird was an adult or a juvenile, or how it died.

Diphacinone residues in birds. Residues of diphacinone were detected in the livers of all Kalij pheasant, Red-billed Leiothrix, and Northern Cardinal, but not in Japanese White-eye captured alive in the treatment plot 1 month after bait application (Table 12, Appendix 25). The highest residue level (4.9 ppm) was found in a Red-billed Leiothrix (# 4021 in Appendix 24). 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.

Invertebrates eating baits. At least 35 species of invertebrates were observed on and/or immediately under Ramik® Green baits (Table 13). The most common were Collembola (possibly more than one species), a large black Carabid beetle (Laemostenus complanatus Dejean, 1828), and the Garlic snail (*Oxychilus alliarius*) (Fig. 4). Carabid beetles formed the greatest biomass on baits, especially at night, and reduced baits to crumbs in a matter of days. Garlic snails and slugs (*Deroceras* spp., *Limax maximus*, and *Milax gagates*) also actively fed on baits, and green bait particles were found in their gut and feces. Slugs characteristically left smooth, slimy hollows on the surface of baits.

G. ENVIRONMENTAL CONDITIONS

The mean daily rainfall recorded for the 5 days after the first bait application (26–30 October) was 3.7 mm, and for the 9 days after the second bait application (31 October–8 November) was 1.0 mm. The average maximum and minimum daily temperatures for the 5 days after the first bait application were 73.0 °F and 51.7 °F, respectively. Temperatures were not recorded for the 9 days after the second bait application (Appendix 12, Deviation 4).

V. DISCUSSION

This study successfully achieved its objectives. Aerial-broadcast application of Ramik® Green bait containing 0.005% (50 ppm) diphacinone, at 22.5 kg/ha (in two applications of 11.25 kg/ha, 1 week apart), was followed by 100% mortality of rats based on radio telemetry results and 99% mortality of rats based on live trapping and non-toxic census bait block interference results. The synchronous death of rats following the application of toxic bait, together with the presence of bait in their stomachs, bleeding characteristic of anticoagulant poisoning, and diphacinone residues in their livers, indicate that the rats likely died of diphacinone poisoning. Most of the toxic bait had disappeared from the forest floor within about 1 month of application. The secondary hazard potential to predatory and scavenging birds from dead rat carcasses was low (because more than 90% of rats died in inaccessible locations). Thus, the results of this study, as with those of the hand-broadcast study (Spurr et al. 2003), demonstrate that Ramik® Green bait containing 50 ppm (0.005%) diphacinone, broadcast at 22.5 kg/ha, is highly effective in reducing populations of rats (predominantly Black Rats) in forest habitat in Hawaii.

As noted by Spurr et al. (2003), the different methods of monitoring the efficacy of toxic bait application had different strengths and weaknesses. Radio-telemetry was the best method, because it enabled the fate of known individuals to be determined. Live-trapping, with ear-

tagging, was also useful but it was not possible to be certain whether the rat without ear-tags captured in the treatment plot 3 weeks after bait application was present before bait application and survived (by not encountering baits, encountering baits but not eating them, or eating insufficient bait), or whether it had only moved into the treatment plot after bait application. The length of time between bait application and live-trapping (3 weeks), the ability of rats to move over distances of several hundred meters within this period of time, and the fact that 44% of the rats captured in the non-treatment plot were recaptures supports the interpretation that it was likely to be an immigrant rather than a survivor of the treatment. Interference to non-toxic census bait blocks was the most difficult method to interpret because of the difficulty of deciding which species (rats, mice, mongooses, birds, or invertebrates) had interfered with the census blocks and deciding what to do about missing census blocks (Spurr et al. 2003). In this study, missing census blocks were deleted from the calculations, and this could have exaggerated the percentage interference to the remaining blocks by less common species. Nevertheless, the reduction in missing census blocks and in rat interference to the remaining census blocks after bait application indicates a successful reduction in rat numbers.

The rats captured in this study were predominantly Black Rats. Insufficient Norway and Polynesian Rats were captured to determine the effect of aerial application of Ramik® Green bait on their population numbers. It has been reported that Polynesian Rats may be less likely than Black Rats to take baits from bait stations (Lindsey et al. 1971; Nelson et al. 2002). However, all Polynesian Rats captured by Dunlevy et al (2000) had eaten placebo Ramik® Green bait hand-broadcast on the ground.

The length of time that bait remained on the forest floor does not appear to have presented a hazard to birds from direct consumption of bait because no mortality of birds likely to have eaten bait was observed. The presence of diphacinone residues in the livers of Kalij Pheasants (up to 0.18 ppm), Red-billed Leiothrix (up to 4.9 ppm), and Northern Cardinals (up to 0.13 ppm), all seed-eating and/or omnivorous birds, indicates that they had eaten bait. However, the concentration of diphacinone found in the livers of these birds is much less than the LD_{50} of diphacinone of 1630 ppm for the Bobwhite Quail (*Colinus virginianus*) and 3158 ppm for Mallard Duck (*Anas platyrhynchos*), the only bird species for which data are available (Tomlin 1994). Bird species so far observed eating Ramik® Green baits are the Kalij Pheasant (Spurr et al. 2002), Erckel's Francolin (*Francolinus erckelli*), Japanese Bush Warbler (*Cettia diphone*), and Red-billed Leiothrix (P. Dunlevy and E. Campbell pers. comm.).

The secondary hazard potential to predatory and scavenging birds (such as the Hawaiian Hawk and Hawaiian Short-eared Owl) from dead rat carcasses was low, because more than 90% of rats died in inaccessible locations. Lindsey and Mosher (1994) reported that avian predators did not take any of the dead (kill-trapped) rats that they placed on the forest floor, but mammalian predators rapidly found and consumed them. Lindsey and Mosher (1994) also reported that radio-collared rats moving during the day, before and after consuming diphacinone bait, remained under cover, minimizing their exposure to avian predators. However, one radio-collared diphacinone-poisoned rat in our study was found moving on the forest floor accessible to avian predators during the day. The Hawaiian Hawk that was found dead in the treatment plot in our study was not found until 6 months after bait application, despite extensive visits to the treatment plot 1 month and 3 months after bait application, and there was no evidence left to

indicate how it died. In general, the risk of secondary poisoning from first-generation anticoagulants such as diphacinone is considered low (Joermann 1998; Eason et al. 2002). For example, in a captive feeding experiment, two out of two Barn Owls survived being fed diphacinone-poisoned rats daily for 10 days (Mendenhall and Pank 1980).

The secondary hazard potential to insectivorous non-target species (birds and bats) from ingestion of invertebrates that have eaten baits was not assessed directly in our study. No insectivorous birds or bats were found dead. Invertebrates that have eaten baits are more likely to form part of the diet of non-target species that scavenge in the forest litter rather than of those that feed from tree trunks or in the canopy. However, even if birds and bats did eat invertebrates that had eaten baits, it is unlikely that they would be able to ingest enough to receive a lethal dose of diphacinone.

By checking baits at night as well as during the day, we found a larger range of invertebrates on baits than Dunlevy et al. (2000) found during the day. Invertebrates that eat Ramik® Green baits are unlikely to be killed by diphacinone because they do not have the same blood clotting systems as vertebrates (Shirer 1992). As well as posing a potential risk of secondary poisoning to insectivorous non-target species, invertebrate consumption of baits also reduces bait availability to rats. However, the extent of this consumption was not sufficient to affect the efficacy of the bait application for rat control.

Rat populations reduced by rodenticides normally recover rapidly by invasion of new rats, normally within 3–12 months (Innes et al. 1995; Nelson et al. 2002; Spurr et al. 2003). Consequently, rodenticides need to be applied continuously or at intervals after the first application. For example, it may be possible to increase the breeding success of some species of birds with annual application of baits for rat control during the peak bird breeding season, of say April–July (Ralph and Fancy 1994).

Future research should investigate the effectiveness of broadcast application of baits for the control of other predators such as Indian Mongooses, and ways of improving the control of House Mice, with the aim of implementing multi-species control for the restoration of forest flora and fauna in Hawaii. Mongooses (with an LD_{50} 0.18 mg/kg) are highly sensitive to diphacinone (Keith et al. 1990), and a recent trial application of 50 ppm diphacinone bait blocks in bait stations was highly effective in reducing mongoose numbers (Smith et al. 2000). House Mouse numbers were reduced by the aerial application of 50 ppm diphacinone bait in this study, 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.

Based on the success of this and previous studies (Lindsey and Mosher 1994; Swift 1998; Dunlevy et al. 2000; Spurr et al. 2003), we recommend that both hand- and aerial-broadcast application of Ramik® Green bait be submitted for registration by the EPA for the control of rat populations in conservation areas in Hawaii.

VI. ACKNOWLEDGMENTS

This study was initiated by the late Gerald Lindsey. Assistance with planning was given by E. Campbell, L.W. Pratt, R. Sugihara, C. Swift, and T. Tunison. We are grateful to the National Park Service for permission to undertake the study in Hawaii Volcanoes National Park; T. Cassie and P. Simmons, Kamehameha Schools, for permission to undertake the placebo trial on Keauhou Ranch; S. Margriter for preparing study site maps; J. Bazzano, I. Cooper, M. Dean, P. Dunlevy, W. Erb, L. Gold, D. Goltz, J. Grossman, B. Issacs, F. Klasner, J. Kunna, A. Least, J. Loda, K. McMurry, S. Munson, J. O'Neill, L.W. Pratt, C. Radford, V. Robbins, N. Shema; H. Sin, B. Spurr, I. Stout, R. Sugihara, and G. Wright for assistance with field and/or laboratory work; B. Ashford for assistance with operating the bait bucket; D. Okida for operating the helicopter; G. Arnold for statistical advice and analysis; S. Anderson, E. Campbell, J. Eisemann, M. Fall, D. LaPointe, C. Natividad Bailey, L.W. Pratt, C. Swift, and T. Tunison for comments on the draft report; C. Bezar for editorial comments; and W. Weller for assistance with word-processing. The U.S. Geological Survey, U.S. Fish & Wildlife Service, and HACCO Inc. provided funding.

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.), Proc. 2nd Hawaii Volcanoes National Park Natural Sciences Conference, Honolulu, USA, pp. 2–5.

Baldwin, P.H. 1941. Checklist of the birds of the Hawaii National Park, Kilauea-Mauna Loa Section, with remarks on their present status and a field key for their identification. Natural History Bulletin No. 7. U.S. Department of Interior, National Park Service.

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

Beauvais, G.P.; Buskirk, S.W. 1999. Modifying estimates of sampling effort to account for sprung traps. *Wildlife Society Bulletin 27*: 39–43.

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, Hawaii, USA.

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

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, USA, pp. 36–51.

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.

Eason, C.T.; Murphy, E.C.; Wright, G.R.G.; Spurr, E.B. 2002. Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. *Ecotoxicology 11*: 35–48.

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.

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.

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

Joermann, G. 1998. A review of secondary-poisoning studies with rodenticides. *Bulletin OEPP/EPPO Bulletin 28*: 157–176.

Johnson, M.S. 1945. Rodent control on Midway Islands. *U. S. Naval Medical Bulletin 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, HI, USA (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.; Nass, R.D.; Hood, G.A. 1971. An evaluation of bait stations for controlling rats in sugarcane. *Journal of Wildlife Management 35*: 440–444.

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.

Malkov, V.; Mach, J.J. 2002. Secondary toxicity examination of avian species on fields treated with 0.005% dipahcinone bait. Genesis Laboratories, Inc., Wellington, CO, USA (unpublished report). 31 pp.

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.G.; 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.

Ralph, C.J.; Fancy, S.G. 1994. Timing of breeding and molting in six species of Hawaiian Honeycreepers. *The Condor 96*:151–161.

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.), Proceedings of the 3rd Conference in Natural Sciences, Hawaii Volcanoes National Park. Honolulu HI, USA, pp. 269–272.

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.

Shirer, M. 1992. In poison's defence. *Terra Nova 17*: 3.

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. 2002. Bait buckets for helicopters. Landcare Research contract report LC0102/62 (unpublished). 23 pp.

Spurr, E.B.; Lindsay, G.D.; Forbes Perry, C.; Foote, D. 2003. Effectiveness of hand-broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests. Unpublished report QA-02, Pacific Island Ecosystems Research Center, Hawaii Volcanoes National Park, HI 96718. 222 p.

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, Hawaii, USA, 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.

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. M.Sc. Thesis, University of Hawaii-Manoa, Honolulu, Hawaii, USA. 92 pp.

Tobin, M.E. 1994. Mitigation of rat depredation in native Hawaiian habitats. *Transactions of the Western Section of the Wildlife Society 30*: 15–20.

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

Tomlin, C. 1994. The pesticide manual. British Crop Protection Council, Farnham, UK. 1341 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.

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

VIII. TABLES

Table 1. Diphacinone content of Ramik® Green baits used in the aerial-broadcast trial, Hawaii Volcanoes National Park, 25 and 30 October 2001.
(Mean and standard deviation).

Date of analysis (month, year)	Age of baits (months)	Diphacinone content (ppm)		Analytical laboratory
		Mean	SD (n)	
September 2000	0	51	Not supplied	HACCO
May 2001	8	49.1	1.2 (6)	Genesis
February 2002	17	52.0	1.1 (4)	Genesis
May 2002	20	50	Not supplied	HACCO

See Appendix 15 and 17 for details.

Table 2. Number of radio-collared rats alive in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Date	Non-treatment plot	Treatment plot	% Change after treatment
Before (22 Oct–24 Oct 01)	18	21	
After (25 Oct–3 Nov 01)	18	0	–100

Table 3. Signs of diphacinone poisoning in radio-collared rats found dead after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001. (Rat 95 was not tested for diphacinone residues because too decomposed).

Rat number			Necropsy results			Diphacinone (ppm) in liver
Tx #	BRD #	LCR #	Internal bleeding	External bleeding	Green-dyed gut	
62	4	4011	+	+	+	2.4
66	3	4010	+	+	+	6.5
95	2	4009	+	+		-
97	6	4013	+	+		12.0
99	8	3989	+		+	4.1
–	1	4008	+		+	0
–	5	4012	+	+		3.5
–	7	3988	+		+	2.1
Total or average			8/8	5/8	5/8	4.4

Tx # is the radio-transmitter number, BRD # is Biological Resources Division number, LCR # is Landcare Research toxicology laboratory number. See Appendix 24 for details.

average w/out 12ppm
=3.1

Table 4. Rat capture rates (rats per 100 corrected trap nights) in live-traps in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Time	Non-treatment plot	Treatment plot	% Change after treatment
Before (16–19 Oct 01)	15.4	20.0	
After 3 weeks (14–17 Nov 01)	10.1	0.2	–98.5
After 3 months (12–15 Feb 02)	4.2	2.8	–48.7
After 6 months (7–10 May 02)	2.9	2.4	–36.3

Table 5. Percentage of rats recaptured after aerial-broadcast application of Ramik® Green bait that had been captured and ear-tagged before application of bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.
(n = number of rats captured).

Time	Non-treatment plot		Treatment plot	
	%	(n)	%	(n)
After 3 weeks (14–17 Nov 01)	44.2	(52)	0.0	(1)
After 3 months (12–15 Feb 02)	54.5	(22)	0.0	(14)
After 6 months (7–10 May 02)	43.8	(16)	0.0	(12)

Table 6. Sex and age classes of rats caught before and after aerial-broadcast application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001. (Sample sizes differ from Table 5 because some rats escaped before they could be sexed or aged).

Time	Sex ratio				Age ratio			
	Non-treatment		Treatment		Non-treatment		Treatment	
	M : F	(n)	M : F	(n)	Ad:Juv	(n)	Ad:Juv	(n)
Before (16–19 Oct 01)	60:40	(65)	54:46	(79)	52:48	(65)	40:60	(79)
After 3 weeks (14–17 Nov 01)	53:47	(47)	100:0	(1)	66:34	(47)	0:100	(1)
After 3 months (12–15 Feb 02)	55:45	(22)	77:23	(13)	95:5	(22)	54:46	(13)
After 6 months (7–10 May 02)	46:54	(13)	80:20	(10)	100:0	(13)	90:10	(10)

M = male, F = female, Ad. = adult, Juv. = juvenile.

Table 7. Rat gnawing on census bait blocks (percentage of those remaining that were gnawed), and percentage change in gnawing in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Time	Non-treatment plot	Treatment plot	% Change in treatment plot
Before (11–12 Oct 01)	46.8	59.4	
After 7 weeks (5–6 Dec 01)	51.1	0.8	–98.8
After 3 months (6–7 Feb 02)	64.8	18.8	–77.1
After 6 months (2–3 May 02)	47.2	19.2	–68.0

Table 8. Mouse captures (per 100 corrected trap-nights) in snap traps, and percentage change in capture rates in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Time	Non-treatment plot	Treatment plot	% Change after treatment
Before (2–3 Oct 2001)	1.19	4.46	
After 1 month (29–30 Nov 2001)	1.09	0.86	–78.9
After 3 months (24–25 Jan 2002)	2.17	5.00	–38.5
After 6 months (25–26 Apr 2002)	0.96	12.50	+247.4

Table 9. Mouse captures (per 100 corrected trap-nights) in live-traps, and percentage change in capture rates in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Time	Non-treatment plot	Treatment plot	% Change after treatment
Before (16–19 Oct 2001)	4.1	11.2	
After 3 weeks (14–17 Nov 2001)	2.1	1.4	–75.6
After 3 months (12–15 Feb 2002)	2.1	8.0	+39.5
After 6 months (7–10 May 2002)	0.4	8.1	+641.3

Table 10. Mouse gnawing on census bait blocks (percentage of those remaining that were gnawed), and percentage change in gnawing in the treatment plot relative to the non-treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

	Non-treatment plot	Treatment plot	% Change after treatment
Before (11–12 Oct 2001)	6.3	30.2	
1 month after (5–6 Dec 2001)	31.9	10.1	–93.4
3 months after (6–7 Feb 2002)	7.4	22.1	–36.0
6 months after (2–3 May 2002)	13.2	21.2	–66.5

Table 11. Locations where radio-collared rats were found dead in the treatment plot after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Rat no.	Recovered	Location
60	Radio collar only	Underground in soil
62	Carcass	Underground in aa
66	Carcass	In dense vegetation
67	Stationary radio signal only	In tree
70	Radio collar only	On ground surface
71	Radio collar only	In dense vegetation
73	Stationary radio signal only	Underground in aa
74	Stationary radio signal only	In tree
76	Stationary radio signal only	In tree
78	Radio collar only	In dense vegetation
80	Stationary radio signal only	In tree
82	Radio collar only	Underground in soil
84	Stationary radio signal only	Underground in aa
87	Radio collar only	Under log
90	Radio collar only	In dense vegetation
94	Carcass	In dense vegetation
95	Carcass	Underground in aa
97	Carcass	In dense vegetation
98	Stationary radio signal only	Underground in aa
99	Carcass	In dense vegetation
100	Radio collar only	Underground in aa

Table 12. Diphacinone residues in the livers of non-target bird species collected alive 1 month after aerial application of 22.5 kg/ha of Ramik® Green bait containing 50 ppm diphacinone, Hawaii Volcanoes National Park, 25 and 30 October 2001.

	Kalij Pheasant	Red-billed Leiothrix	Northern Cardinal	Japanese White-eye
Number sampled	2	8	2	5
% positive diphacinone	100	100	100	0
Mean ppm diphacinone	0.15	2.45	0.11	0
Range ppm diphacinone	0.12–0.18	0.74–4.9	0.08–0.13	0–0

See Appendix 17, 24, and 25 for details.

Table 13. Invertebrate species observed on and/or immediately under Ramik® Green bait containing 50 ppm diphacinone, Hawaii Volcanoes National Park, October–November 2001.

Class	Order	Family	Genus and species
Oligochaeta			Earthworm sp.
Gastropoda	Stylommatophora	Zonitidae	*Oxychilus alliarius* (garlic snail)
		Agriolimacidae	*Deroceras laeve*
		Agriolimacidae	*Deroceras reticulatum*
		Agriolimacidae	*Deroceras* sp.
		Limacidae	*Limax maximus*
		Milacidae	*Milax gagates*
Turbellaria	Tricladida		Flatworm sp.
Crustacea	Isopoda	Porcellionidae	*Porcellio scaber*
	Amphipoda		Amphipod sp.
Arachnida	Araneida		Spider sp.
	Acarina		Mite sp.
Myriapoda	Diplopoda		Large millipede sp.
			Small millipede sp.
	Chilopoda		Centipede sp.
Collembola	Collembola	Entomobryidae	Springtail sp.
Insecta	Dermaptera	Forficulidae?	Earwig sp.
	Orthoptera	Acrididae	Grasshopper sp.
		Gryllidae	Cricket sp.
	Hemiptera	Lygaeidae	Seed bug sp.
	Coleoptera	Carabidae	*Laemostenus complanatus*
		Carabidae	Small brown beetle sp.
		Carabidae	Medium brown beetle sp.
		Elateridae	Click beetle sp.
		Nitidulidae	Sap beetle sp.
		Staphylinidae	Rove beetle sp.
	Diptera	Tipulidae	Crane fly sp.
		Drosophilidae	Fruit fly sp.
		Phoridae	Phorid sp.
		???	Fly sp.
	Lepidoptera		Lepidopteran sp. (larva)
			Lepidopteran sp. (larva)
			Lepidopteran sp. (larva)
	Hymenoptera	Formicidae	Ant sp.
	Psocoptera	Psocidae	Bark louse sp.

IX. FIGURES

Figure 1. Helicopter bait bucket used for aerial application of Ramik® Green bait in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 25 and 30 October 2001

Figure 2. Flight path of helicopter recorded by Garmin GPS during aerial application of the first and second swathe of Ramik® Green bait in relation to proposed flight path over the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 30 October 2001

Figure 3. Disappearance rate of Ramik® Green bait (expressed as % baits remaining on the forest floor) after aerial application in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 25 and 30 October 2001

Figure 4. Invertebrates observed on Ramik® Green baits in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, October–November 2001

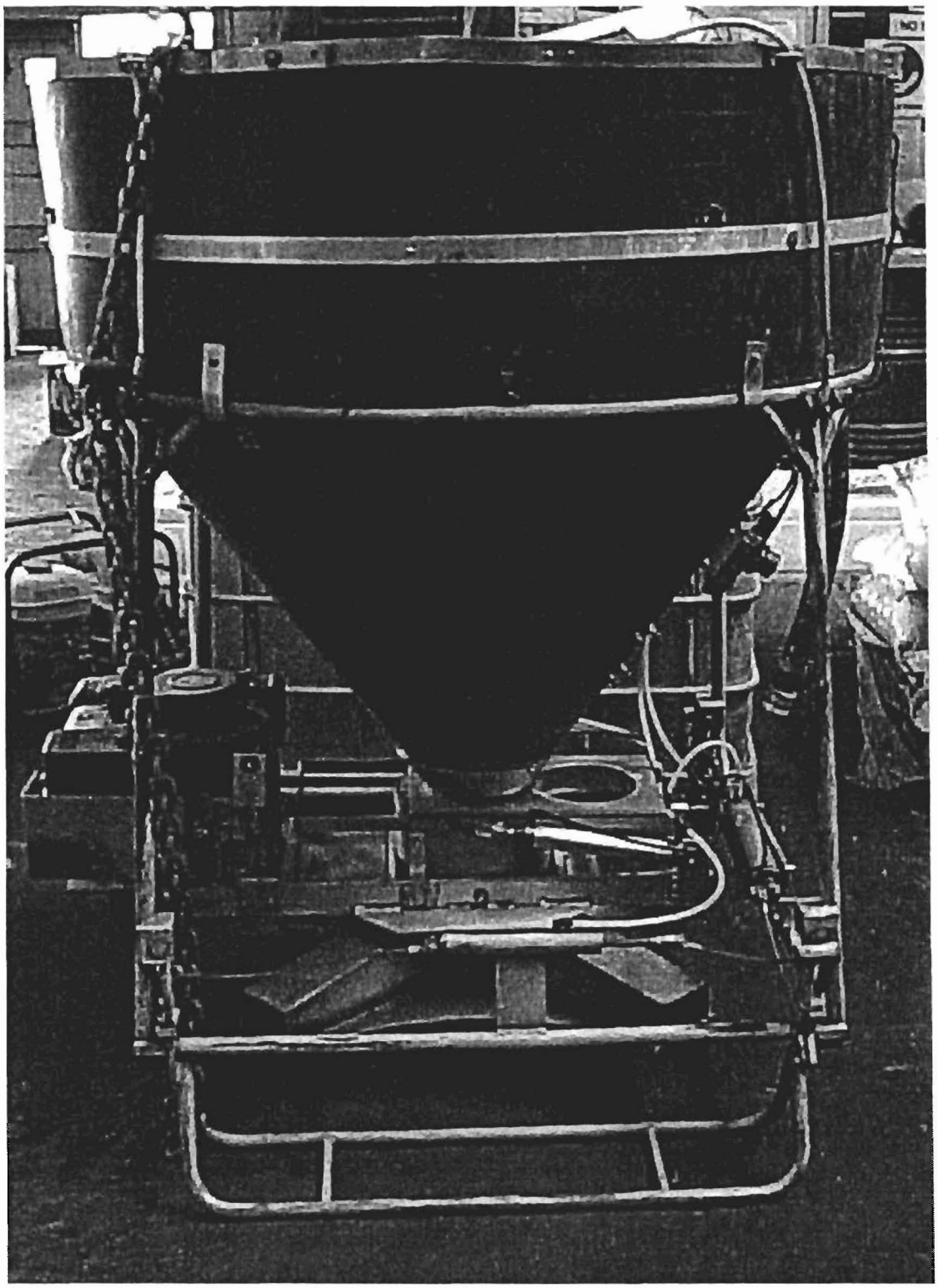


Figure 1. Helicopter bait bucket used for aerial application of Ramik® Green bait in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 25 and 30 October 2001.

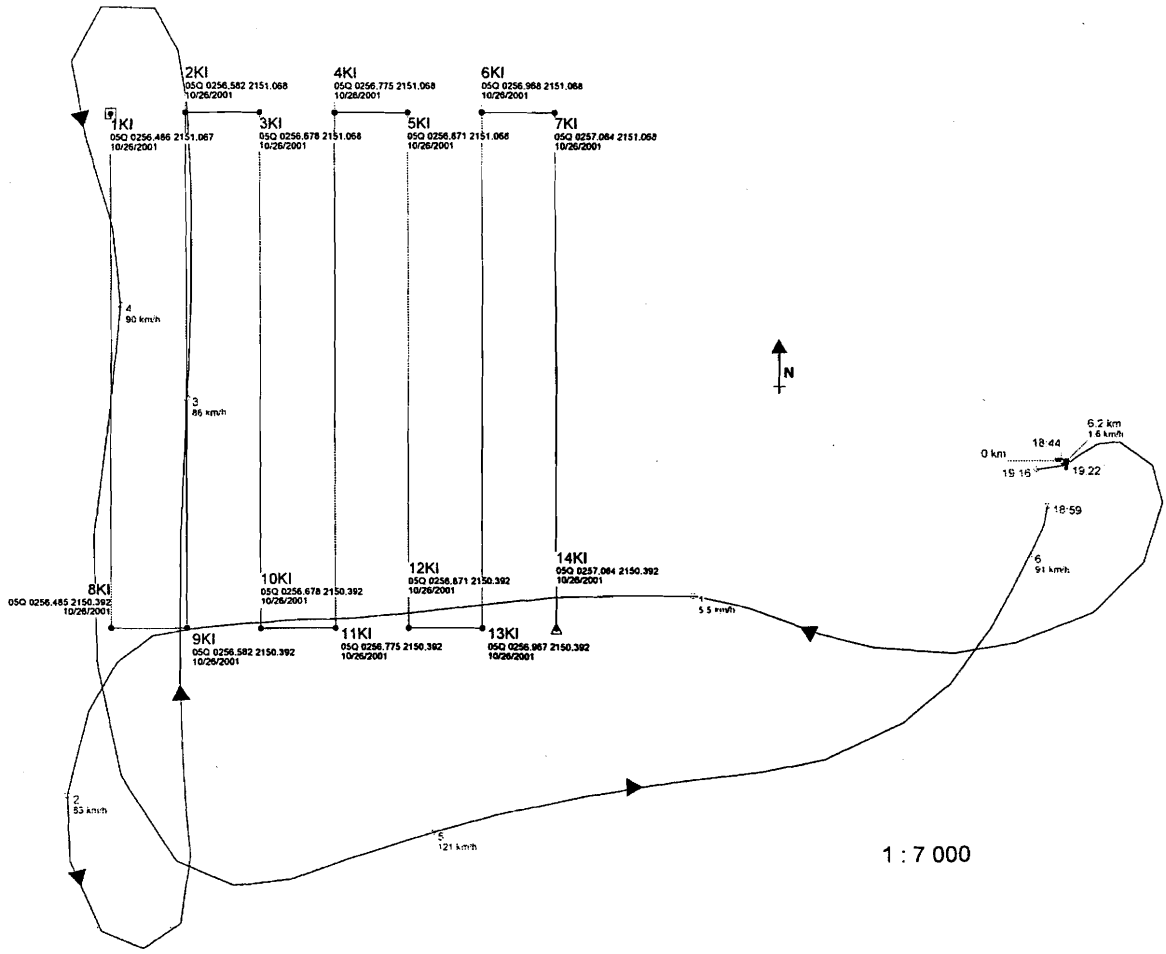


Figure 2. Flight path of helicopter recorded by Garmin GPS during aerial application of the first and second swathe of Ramik® Green bait in relation to proposed flight path over the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 30 October 2001.

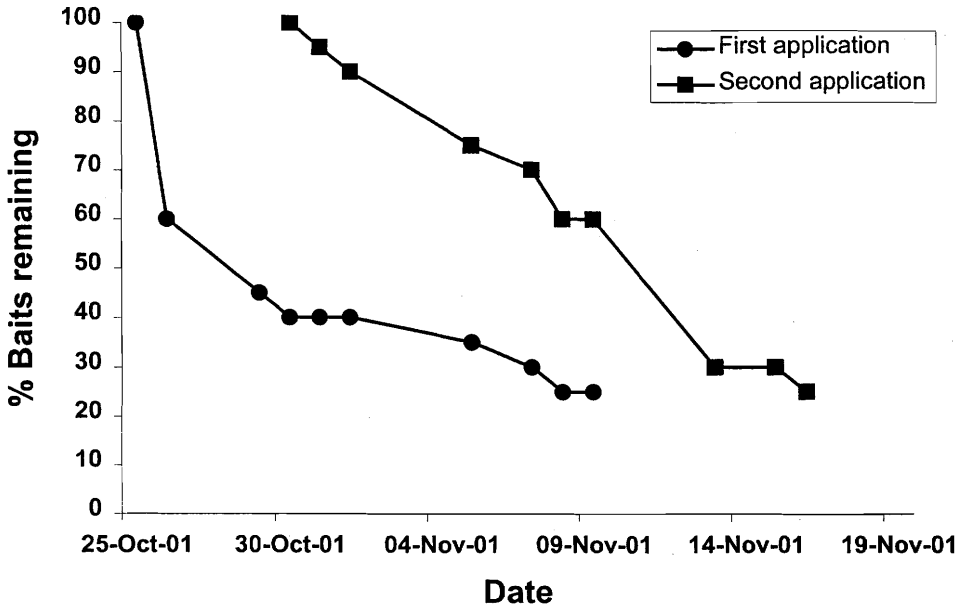


Figure 3. Disappearance rate of Ramik® Green bait (expressed as % baits remaining on the forest floor) after aerial application in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, 25 and 30 October 2001.

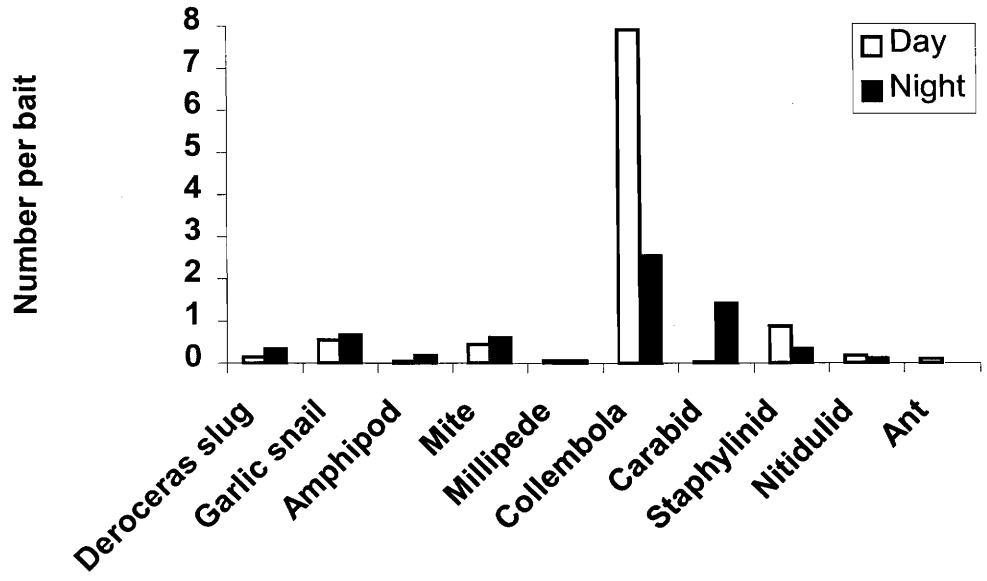


Figure 4. Invertebrates observed on Ramik® Green baits in the treatment plot, Kipuka Ki, Hawaii Volcanoes National Park, October–November 2001.

X. APPENDICES

Appendix 1. Experimental Use Permit.
Appendix 2. Concurrence letter from U.S. National Park Service.
Appendix 3. Concurrence letter from U.S. Fish and Wildlife Service.
Appendix 4. Concurrence letter from State of Hawaii Division of Forestry and Wildlife.
Appendix 5. Concurrence letter from The Nature Conservancy of Hawaii.
Appendix 6. Biological opinion from U.S. Fish and Wildlife Service.
Appendix 7. Amendment to biological opinion from U.S. Fish and Wildlife Service.
Appendix 8. Environmental assessment report.
Appendix 9. National Park Service acceptance of environmental assessment report.
Appendix 10. Application for Experimental Use Permit.
Appendix 11. Amendments to protocols in EUP application.
Appendix 12. Deviations from protocols in EUP application.
Appendix 13. Ramik® Green label.
Appendix 14. Material safety data sheet.
Appendix 15. HACCO certificates of analysis of Ramik® Green bait.
Appendix 16. End-use product tracking form for Ramik® Green bait.
Appendix 17. Malkov and Mach (2002), Genesis Laboratories report.
Appendix 18. Flow rate of Ramik® Green bait through the helicopter bait bucket.
Appendix 19. Swathe width of Ramik® Green bait applied by the helicopter bait bucket.
Appendix 20. Bait size distribution before and after aerial application.
Appendix 21. Accuracy of aerial application of toxic bait in the treatment plot.
Appendix 22. Standard Operating Procedures.
Appendix 23. Timetable of monitoring activities.
Appendix 24. Landcare Research toxicology laboratory analysis report.
Appendix 25. Diphacinone residues in birds.

Appendix 1. Experimental Use Permit.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 25, 2000

Biological Resources Division
US Geological Survey
Pacific Island Ecosystems Research Center
P.O. Box 44
Hawaii National Park, HI 96718

Attention: Dr. David Foote

Subject: Experimental Use Permit No. 73815-EUP-1
Approval of Experimental Use Permit (EUP)
to Control Introduced Rat Populations
Your Application for Experimental Use of May 1, 2000
Quantities Authorized: 3,000 Pounds of 0.005% Bait or 0.15 Pounds of
Diphacinone as Active Ingredient
Acreage Authorized: 120 Acres
Effective Dates: January 24, 2001 to January 24, 2002

Based upon information submitted with the above letter, the Agency is hereby approving the above experimental use permit for one year, under section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (86 Stat. 983). Ramik Green rodent bait, manufactured by Hacco, Inc. of Madison, WI 53707 will be used in two kipukas (forested area) to control commensal rats and other introduced species. Killing rats is expect to enhance the survival prospects of threatened and endangered types of plants and animals. The Agency considers this EUP to be a non-food, non-feed use, as set forth below.

Shipment and/or use under this permit is subject to the provisions of 40 CFR 172.

PRIOR TO SHIPMENT AND/OR USE OF THIS MATERIAL, YOU MUST CONSULT WITH THE STATE PESTICIDE REGULATORY OFFICIALS OF THE STATES IN WHICH YOUR EXPERIMENTAL PROGRAM WILL BE CONDUCTED AND OBTAIN A STATE PERMIT OR LICENSE IF SUCH IS REQUIRED. ISSUANCE OF THIS FEDERAL PERMIT DOES NOT NEGATE THE NEED FOR PERMISSION FROM INDIVIDUAL STATES. IN ADDITION, PRIOR TO INITIATION OF THIS EXPERIMENTAL PROGRAM IN AN STATE, YOU ARE TO NOTIFY THE STATE

LEAD AGENCY OF THE STATES IN WHICH YOUR EXPERIMENTAL PROGRAM WILL BE CONDUCTED OF THE SPECIFIC TESTING PROGRAM (WHEN, WHERE, HOW MUCH, ETC.). FAILURE TO DO SO MAY RESULT IN REVOCATION OR MODIFICATION OF THIS EXPERIMENTAL USE PERMIT.

Based upon the experimental program submitted, this product may be shipped for use under this permit to the following states in the amounts set forth in the following table.

Regions and States of Use Acreage and Pounds of Active Ingredient (Diphacinone) and 0.005% Bait Authorized for 73815-EUP-1		
Region and State	Acreage	Pounds of Active Ingredient
A. Region 9		
1.Hawaii	120	0.15 Pounds (3000 Pounds of 0.005% Bait)
TOTALS	120	0.15 Pounds (3000 Pounds of 0.005% Bait)

The subject product will be applied subject to the following conditions:

1) Follow the labeling accepted for this product today, January 24, 2001, which is applicable for this experimental use permit. Prior to registration of this product under section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) , you will need to address any outstanding data requirements.

2) Revise the use directions on the proposed experimental use permit (EUP) label to read as shown below.

DIRECTIONS FOR USE

Use of this product in a manner inconsistent with the terms of this Experimental Use Permit is a violation of Federal Law.

USE RESTRICTIONS: This product may be used experimentally to control roof rats (*Rattus rattus*), Polynesian rats (*R. exulans*), Norway rats (*R. norvegicus*) and house mice (*Mus musculus*) on Kipuka Ki on Mauna Loa on the Island of Hawaii. Use of this product must be supervised by Dr. David Foote, Kilauea Field Station, Biological

Resources Division (BRD), U.S. Geological Survey (USGS). This product was manufactured at the request of the USGS/BRD, Kimauea Field Station, Hawaii National Park; and the National Park Service, U.S. Department of the Interior, Hawaii Volcanoes National Park. Refer to the program outline in the application for this experimental use permit (2393-EUP-1), as modified by EPA's acceptance letter and this label, for additional details on the use of this product.

APPLICATION DIRECTIONS: Broadcast bait pellets aerially by helicopter on manually at a rate of 10-12.5 lbs of bait per acre per treatment. Five to seven days after the first application, make a second broadcast application at a rate no higher than 12.5 lbs of bait per acre. Do not exceed a cumulative application rate of 25 lbs of bait per treated acre.

3). For a §24(c) registration, the **"DIRECTIONS FOR USE"** section should be more complete and explicit than for an experimental use permit, and should clearly indicate what is required of users. We suggest the text shown below (assuming that the baiting procedures and rates proposed for this EUP turn out to be appropriate and that we have reflected jurisdictional authorities appropriately).

DIRECTIONS FOR USE

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

USE RESTRICTIONS: This product may only be used to control roof rats (*Rattus rattus*), Polynesian rats (*R. exulans*), Norway rats (*R. norvegicus*), house mice (*Mus musculus*), and small Indian mongoose (*Herpestes auropunctatus*) in forests, offshore islands, national parks, refuges, and other reclamation areas to protect native threatened or endangered species of plants and animals. Use of this product must be approved by the U.S. Department of Interior (USDI) and the Hawaii Department of Land and Natural Resources (HDLNR). All applications must be made or directed by a Federal or State agency authorized to use this product.

Do not use this bait in areas occupied by the endangered Hawaiian crow (Kona area on the Island of Hawaii) unless specific approval is obtained from the USDI and HDLNR.

Do not apply this product in or around crops grown for human food or animal feed. Do not graze livestock in treated areas. Do not use this

product on any site where the control objective could be met by applying rodenticides in secured, tamper-resistant bait stations.

Report any signs of secondary poisoning of animals other than target rodents or small Indian mongoose to the Pesticides Branch of the Hawaii Department of Agriculture within 24 hours of discovery.

APPLICATION DIRECTIONS: Broadcast bait pellets aerially by helicopter on manually at a rate of 5 to 10 lbs of bait per acre per treatment. Retreat area at 5 to 10 lbs of bait per acre 5 to 7 days after first treatment. If rat activity persists after second treatment and rodents continue to accept bait, make hand broadcast applications in areas where there is residual rat activity. Do not exceed a cumulative application rate of 25 lbs of bait per treated acre.

Check treated and surrounding areas periodically for signs of dead animals. Collect and dispose of animal carcasses, unless instructed by a State or Federal agency to have carcass retained for evaluation. Dispose of dead animals at a sanitary landfill or by burial on site. Burial on site shall be at a depth such that nontarget vertebrate animals will not gain access to the carcass.

---

4) final report will be required at the end of the experimental program, including all items in 40 CFR 172.8(b).

If you have any questions about this approval, please contact Mr. Dan Peacock by phone (703-305-5407), fax (703-305-6596), or E-Mail (peacock.dan@epa.gov).

Sincerely,

Meredith Laws, Acting Branch Chief
Insecticide-Rodenticide Branch
Registration Division (7504C)

NOT FOR SALE
FOR RESEARCH PURPOSES ONLY
SAME FORMULATION AS: **RAMIK GREEN**, EPA Reg. No. 2393-498

for experimental purposes under the provisions of the Federal Insecticide Act, subject to attached comments.
Permit No. 73815-EUP-1
Issued on 1-25-2001

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 K1, 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.

DIRECTIONS FOR USE:
Use of this product in a manner inconsistent with the terms of this Experimental Use Permit is a violation of Federal Law.

USE RESTRICTIONS: This product may be used experimentally to control roof rats (Rattus rattus), Polynesian rats (R. exulans), Norway rats (R. norvegicus) and house mice (Mus musculus) on Kipuka Ki on Mauna Loa on the Island of Hawaii. Use of this product must be supervised by Dr. David Foote, Kilauea Field Station, Biological Resources Division (BRD), U.S. Geological Survey (USGS). This product was manufactured at the request of the USGS/BRD, Kilauea Field Station,

Hawaii National Park; and the National Park Service, U.S. Department of the Interior, Hawaii Volcanoes National Park. Refer to the program outline in the application for this experimental use permit (~~73815-EUP-1~~), as modified by EPA's acceptance letter and this label, for additional details on the use of this product.

APPLICATION DIRECTIONS: Broadcast bait pellets aerially by helicopter on manually at a rate of 10-12.5 lbs of bait per acre per treatment. Five to seven days after the first application, make a second broadcast application at a rate no higher than 12.5 lbs of bait per acre. Do not exceed a cumulative application rate of 25 lbs of bait per treated acre.

Applicant: Biological Resources Division
US Geological Survey
Pacific Island Ecosystems Research Center
P.O. Box 44
Hawaii National Park, HI 96718

Bait manufactured by: HACO, INC., P.O. BOX 7190, MADISON, WI 53707

NET CONTENTS: 50 pounds (22.68 Kg.)
CODE L00

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.

HACO, INC.•P.O. BOX 7190•MADISON, WI 53707
NET CONTENTS: 50 pounds (22.68 Kg.)
CODE L/00

Appendix 2. 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 3. 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 4. 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 5. Concurrence letter from The Nature Conservancy.

The Nature
Conservancy
of Hawai'i
[illegible]anu Avenue
[illegible]i, Hawai'i 96817
Phone (808) 537-4508
Facsimile (808) 545-2019

The Nature Conservancy®
ational
uarters
245 N. Fairfax Dr., Suite 100
rlington, VA 22203-1606
ttp://www.tnc.org

The Nature Conservancy of Hawai'i

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 6. 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 5, 1984. 48 pgs.

Appendix 7. Amendment to 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 Blvd, Rm 3122
Box 50088
Honolulu, HI 96850

OCT 10 2000

In Reply Refer To: 1-22-99-F-03 (JTN)

Memorandum

To: Chief of Resources Management, Hawaii Volcanoes National Park

From: Paul Henson, Field Supervisor, Pacific Islands Office, U.S. Fish and Wildlife Service, Honolulu, Hawaii

Subject: Amendment of Section 7 Consultation on Experimental use of Diphacinone Pellets in Hawaii Volcanoes National Park (Biological Opinion Number 1-2-99-F-03).

This memorandum constitutes an amendment to the August 6, 1999, Biological Opinion (BO) on the experimental use of diphacinone pellets in Hawaii Volcanoes National Park (HAVO) (File No.: 1-2-99-F-03). The Fish and Wildlife Service received a letter dated August 10, 2000, from Chief of Resources Management, HAVO, requesting amendment of the BO to allow aerial broadcasting of diphacinone pellets over a 112-acre site in Kīpuka Kī, near Mauna Loa Road in HAVO. Our review of the amendment request indicates the total area over which diphacinone will be applied in Kīpuka Kī is approximately eleven times that of the ten acre area for which hand-broadcast application of diphacinone was permitted in the BO.

It was concluded in the BO that effects of hand-broadcast application of diphacinone, as proposed, were not likely to jeopardize the continued existence of the `io (*Buteo solitarius*). Results of studies on the effects of the action on listed species suggested risk of secondary poisoning of `io were extremely low because of rodent and `io behaviors, the short length of time in which diphacinone contaminated rats would be available for scavenging, the availability of a wide range of prey to `io, the limited size of the treatment area, and the low densities of `io in the treatment area. As stated in the request for amendment, no untoward non-target effects have been detected for hand-broadcast applications of diphacinone at Kīpuka Kī or `Ola`a forest, a second ten acre area where hand-broadcast application of diphacinone was performed.

Although the treatment area will be larger at Kīpuka Kī, there is no anticipated change of the effects of the action on listed species or cumulative effects, because of the relatively small area over which diphacinone will be applied in relation to territory size of `io, and the reasons listed above. Amount or extent of take, effect of take, and reasonable and prudent measures under the incidental take statement in the BO remain the same. All terms and conditions in the BO will continue to be observed.

Accordingly, permission is granted to conduct two aerial drops of diphacinone pellets over 112 acres in Kīpuka Kī , about a week apart at 10lbs/ac each.

If you have any questions, please contact Fish and Wildlife Biologist, Jay Nelson, by telephone at (808) 541-3441 or by facsimile transmission at (808) 541-3470.

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

Appendix 8. Environmental assessment report.

ENVIRONMENTAL ASSESSMENT: TEST BROADCAST RODENTICIDE RANIK 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 `Elepaio increased in an experimental control program on O`ahu 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 Hawai`i. 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 Kī 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 Kī. 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 KIpuka Puaulu and a nearby area of `Ōlà`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 `Io 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.

Kīpuka 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 Kī. 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 `Ïo 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 `Ïo, 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 Ki 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

Dr. David Foote, Biological Resources Division, US Geological Survey

Dr. Gerald Lindsey, Biological Resources Division, US Geological Survey

Laura Schuster, Cultural Resources Specialist, Hawaii Volcanoes National Park

Gary Barbano, National Park Service Planner, Pacific Islands Support Office, Honolulu

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. giffardianus*; 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 9. National Park Service acceptance of environmental assessment report.

United States Department of the Interior

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

IN REPLY REFER TO:

L7617 (PGSO-PP) OCT 05 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. Lee Acting

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

QA-02

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

Q4-02

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. Lucas for Date 10/5/99
Director
Pacific West Region

QA-02

Appendix 10. Application for experimental use permit.

April 19, 2000

Application for experimental use permit to ship and use a pesticide for experimental purposes only.

1. General Requirements:

(a) (1) Application for Experimental Use Permit, EPA Form 8570-17.

(a) (2) EPA Reg. No. 2393-498, EPA Est. No. 61282-WI-I

(a) (3) Introduction, purpose and objectives of proposed testing

Alien small mammal and invertebrate predators have had devastating impacts on insular environments worldwide (Atkinson 1977, Buckle and Fenn 1992, Moors et al. 1992, Seto and Conant 1996). In Hawaii, evolution of the fauna and flora 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. 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 rat (*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, Lindsey et al. 1999). These rodents, together with the introduced feral cat (*Felis catus*) and Indian mongoose (*Herpestes auropunctatus*) inhabit forest habitat in varying degrees of sympathy with native Hawaiian forest birds, plants, and invertebrates (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 also known predators to ground and burrow nesting birds (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 Island, 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).

There are only two methods (trapping and bait stations) 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 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 the 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 (USGS/BRD, unpubl. data).

Purpose: To develop a safe, economical method to control introduced rats in noncrop conservation areas of Hawaii.

Objective: To determine efficacy of aerial broadcast baiting with a 0.005% diphacinone pellet bait, to determine disappearance rate from the forest floor of diphacinone bait pellets, and to monitor secondary hazard potential from aerially broadcast baiting 0.005% diphacinone bait pellets.

(a) (4) A description in detail of the proposed testing program including:

Test parameters:

Study area: Two 45.3 ha (112 acres; 673 x 673 m) study plots will be established in a mesic forest at Kipuka Ki (treatment) and Kipuka Puaulu (control) within Hawaii Volcanoes National Park on the island of Hawaii, State of Hawaii. Kipuka Ki is separated from Kipuka Puaulu by 1.53 km. Neither study area contains surface water impoundments or streams. No buildings or other man-made structures are located within 1 mile of the treatment study site.

Livetrapping: Within the center of each 45.3 ha plot, 144 basket live traps will be placed at 25 m intervals on transects spaces 25 m apart to obtain indices of rat densities pre- and post-treatment. 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. Between trapping periods, traps will remain in a closed position at each trap location. Traps will also be opened 2 weeks before aerial baiting, and 2 weeks, 2 months, and 4 months after aerial bait application to determine efficacy and rat re-invasion rates. For each trapping period, trap locations 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. 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.

Census blocks. One week before each treatment plot is baited, 132 non-toxic census bait blocks will be placed on a 25- x 25-m spacing within each 45.3-ha plot. Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5m, 62.5m, etc.) for 2 nights before baiting, and at 3 weeks, 9 weeks and 17 weeks 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 bait blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared using Chi-square analysis.

Radio Telemetry. Before the initial bait application, up to 25 rats within the treatment and up to 25 rats within the control plot will be fitted with transmitters. Each rat will be monitored daily for at least 3 consecutive nights immediately before baiting to ensure the rats are active and alive. 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. 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. The proportion of radio-collared rats surviving in post-treatment within the treatment and control plots will be compared by Chi-square analysis.

Diphacinone Baiting. One fish-flavored formulation (Ramik® Green, HACCO, Inc., Madison, WI) of 0.005% diphacinone pellet baits (6 g/pellet) will be tested. (The bait to be tested is the same formulation as the registered Ramik Green bait [EPA Reg. No. 2393-498], except it is prepared in a 6 g pellet.) The 45.3 ha treatment plot will be aerial broadcast baited with the fish-flavored diphacinone bait. The other 45.3 ha plot will not be baited to serve as a control. One-half of the bait (11.5 kg/ha; 10 lbs./acre) will be distributed aerially on Day 1 and the other half (11.5 kg/ha; 10 lbs./acre) will be distributed on Day 5-7. This application schedule is to ensure that bait pellets will be available to rats for 10-15 consecutive days as recommended by Dunlevy et al (2000; Attachment C). The total amount of bait aerially distributed will be 22.5 kg/ha (20 lbs./acre). Bait pellets will be aerially applied when the ground is reasonably dry and weather predictions call for fair weather in the next 5 days.

Bait Disappearance rate. Immediately after the first 11.25 kg/ha bait application, 20 individual bait pellets will be randomly located within the center 4 hectares of the 45.3 ha

treatment plot. A 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 the bait is taken or the bait disintegrates. Data will be recorded on disappearance, feeding by slugs, snails and other invertebrates, and consumption by rats.

Analytical chemistry. The concentration of diphacinone in the baits will be analyzed by Genesis Laboratories, Inc., Wellington, CO.

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 bait distribution.

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 randomly selected transects (673 m long x 5 m wide and spaced at least 50 m apart; 3% 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. Any carcass discovered will be recorded to species, weighed, sexed, placed in individually marked containers, frozen and retained for possible residue analysis.

Designation of the pest organisms involved:

Target species include the black rat (*Rattus rattus*), Polynesian rat (*R. exulans*), and Norway rat (*R. norvegicus*).

Amount of pesticide product proposed for use:

22.5 kg/ha (20 lbs./acre). 1019.25 kg (2240 pounds) total.

The crops, fauna, flora, sites, modes, dosage rates and situation of application:

Kipukas Puaulu and Ki, ancient areas of deep ash soil surrounded by lava of the late prehistoric Keamoku flows are contained within a mesic forest within Hawaii Volcanoes National Park. Both kipukas are on the lower slope of Mauna Loa at 1,200-1,360 m elevation. Kipuka Ki is separated from Kipuka Puaulu (control site) by 1.53 km.

Vegetation within the central part of the kipukas is composed of a tall koa/`ohi`a/soapberry (*Acacia koa/Metrosideros polymorpha/Sapindus saponaria*) forest. Native ferns and herbs dominate ground cover 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*). 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. Neither area contains surface water

impoundments or streams. No buildings or other manmade structures are located within 1 mile of the treatment study site.

Fauna include seven endemic bird species -- the elepaio (*Chasiempsis sandwichensis*), omao (*Myadestes obscurus*), apapane (*Himatione sanguinea*), iiwi (*Vestiaria coccinea*), Hawaii amakihi (*Hemignathus virens*), and Hawaiian hawk (*Buteo solitarius*); and six introduced bird species – northern cardinal (*Cardnalis cardinalis*), red-billed leiothrix (*Leiothrix lutea*), kalij pheasant (*Lophura leucomelana*), melodious laughing thrush (*Garrulax canorus*), Japanese white-eye (*Zosterops japonicus)*, and house finch (*Carpodacus mexicanus*). Mammals found within the study area include four rodent species (*Rattus rattus, R. exulans, R. norvegicus*, and *Mus musculus*), the feral cat (*Felis catus*) and the Indian mongoose (*Herpestus auropunctatus*).

The mode of dispersal and the dispersal rate will be a 100-m swath out of a Dryslinger II bucket slung under a Hughes 500 helicopter, sowed on two separate days at the rate of 11.2 kg/ha (10 pounds per acre) per day. The two aerial broadcast baiting applications, separated by 5 – 7 days, is to ensure that bait pellets are available to rodents for 10-15 consecutive days as recommended by Dunlevy et al. (2000; Appendix C). Total amount of bait distributed will be 22.5 kg/ha (20 lbs./acre).

The treatment site is a 45.3 hectare (673m x 763m) area of Kipuka Ki (elevation between 1200- and 1270-m) located in a mesic forest within the Hawaii Volcanoes National Park, Island of Hawaii, State of Hawaii. The top edge of the site is located 250 m below (south) of Mauna Loa Strip Road that transects the center of Kipuka Ki (See map – Attachment A).

Testing will be conducted starting January 2001 and be completed by January 2002.

The manner in which supervision of the program will be accomplished.

The program will be supervised by Dr. David Foote, Ecologist, Pacific Island Ecosystems Research Center, Kilauea Field Station, P. 0. Box 52, Hawaii National Park, HI 97818.

(a) (5) Name, address, telephone number, and qualifications of all participants

Dr. David Foote, Ecologist
USDI/Biological Resources Division
Pacific Island Ecosystems Research Center
P. O. Box 52
Hawaii National Park, HI 96718
Phone: (808) 985-6070

- Dr. Foote obtained his doctorate in Ecology from the University of California at Davis. He has nine years experience in assessment and restoration research of native invertebrates and vertebrates in Hawaiian ecosystems. His research interest includes assessment and restoration of host-plant associated arthropod biodiversity; effects of introduced mammals on community structure of native and non-indigenous invertebrates; relationship between ecosystem function and biological diversity; impact and control of

alien social hymenoptera in Hawaii; classical biological control of invasive weeds; and phenotypic evolution of insects following historical colonization events.

Dr. Eric Spurr
Landcare Research
P.O. Box 69, Lincoln
New Zealand
New Zealand phone: +64 (03) 325 6705
Hawaii phone: (808) 985-6070

- Dr. Spurr obtained his doctorate in Zoology from the University of Canterbury, New Zealand. He has 27 years of research experience. Since 1992 his research projects include developing toxic baits for wasp control, developing toxic baits for rodent and mustelid control, assessing impacts of pest control on non-target species, and developing repellents to protect non-target species from pesticides. Other experience include leader of multi-disciplinary research teams, comprising up to six scientists and technical staff, spanning entomology, toxicology, and vertebrate ecology.

Charlotte Forbes
USDI/Biological Resources Division
Pacific Island Ecosystems Research Center
P. O. Box 44
Hawaii National Park, HI 96718
Phone: (808) 967-7396 ext. 240

- Ms. Forbes has a Bachelors of Science degree in Wildlife Resources from the University of Idaho. She has 15 years experience as a research assistant and/or wildlife technician working on various conservation projects, including the evaluation of diphacinone for the control of mongoose and rats in conservation areas of Hawaii. Beginning in 1999, she has been the lead field technician evaluating hand broadcast baiting using a 0.005% diphacinone bait pellet for the control of rats in mesic and wet forests of Hawaii.

(a) (6) Name and address of all cooperators

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

Tim Tunison
Hawaii Volcanoes National Park.
Chief of Resource management
P.O. Box 52
Hawaii National Park, HI 96718

Thomas L. Hauptman
President, Pacific Helicopter Tours, Inc.
Kahului Heliport, Hanger 109
Kahului, HI 96732

Pacific Helicopters are certified in aerial applications of agricultural pesticides and will use a Hughes 500 to apply the bait.

(a) (7) Information on prior testing

1. In standard laboratory feeding bioassays with commercial Ramik® Green pelletized bait containing 0.005% diphacinone, Swift (1998; Attachment B) determined that minimum exposure times and bait amounts of seven days and 37.5 g (15 commercial-size pellets) for *Rattus rattus,* and six days and 30.0 g (12 commercial-size pellets) for *R. exulans* were required to achieve EPA acceptable control of wild caught rats from Hawaiian ecosystems.

2. In a broadcast bait application rates' study using Ramik® Green placebo bait pellets (formulated without diphacinone) and coated with a biological tracer, Dunlevy et. al (2000; Attachment C) evaluated three application rates (11.5, 22.5 and 33.75 kg/ha) to determine the optimal broadcast application rate that will result in maximum exposure to rodents in Hawaiian ecosystems. Baits were also monitored for degradation to ascertain that baits would be available to rats for the necessary exposure time of 7 days required to reach the lethal dosage determined in laboratory trials (Swift 1998). Results indicate that an application rate of 22.5 kg/ha would maximize bait exposure to rats, while minimizing excess bait usage. Ninety-four percent and 100% of *Rattus rattus* and *R. exulans,* respectively, were known to have consumed bait pellets at this application rate. These results were 23% higher than the 11.5 kg/ha application rate and only 2% lower than the 33.75 kg/ha application rate. Dunlevy et. al recommended a minimum bait life after application of 10-15 days (4-8 days for rats to become accustom to a new type of food plus 6-7 consecutive days of bait consumption). Ramik Green pellets meets this criterion.

3. In a hand broadcast baiting study using 0.005% diphacinone bait pellets (Ramik® Green; 6g pellet), Lindsey and Forbes (2000; Attachment D) obtained 100% control of the rat population in two 4-ha study areas (wet forest and mesic forest) within the Hawaiian ecosystem. Bait application rate was 22.5 kg/ha with one-half (11.25 kg/ha) of the bait pellets distributed on Day 1 and one-half (11.25 kg/ha) on Day 5. Bait pellets were applied twice to ensure that fresh bait pellets were available for 10-15 consecutive days as recommended by Dunlevy et.al (2000; Attachment C).

4. In 1999, Tonnie Casey of Kamehameha Schools/Bishop Estate (a large private landowner) conducted an experimental drop of an Eaton's fish-flavored 8-gram wax-based pellet for the control of rats in Hawaii. She used a sowage rate of 15 lbs/acre over

a 70-acre area at 6,200-ft elevation in a wet forest habitat. Preliminary results from this study suggested good control of the rats with the 20 rats marked with radio collars dying within 6 days after the bait drop (Attachment E, pages 2-3 in draft minutes of Hawaii Toxicant Working Group Meeting August 26, 1999).

5. Lindsey and Mosher (1994: Attachment F) assessed the secondary hazard potential of diphacinone to non-target avian predators, particularly the Hawaiian hawk or Io and the Hawaiian owl or Pueo (*Asio flammeus*), within forested areas of Hawaii. Dead rats (kill trapped) were placed on the forest floor to determine scavengers taking them. Mammalian predators rapidly located (mean of 2.9 days) and consumed the dead rats. Avian predators did not locate or take any of the exposed, dead rats. Radio-marked rats moving 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 owls as a result of poisoning from diphacinone was considered minimal.

(a) (8) Proposed method of storage and disposition of unused experimental pesticide and its containers.

Bait pellets will be stored in plastic bags enclosed within cardboard boxes within a locked storage room within Building 216, Hawaii Volcanoes National Park. Building 216 is a combination office/laboratory/storage building, which is locked each night after working hours.

Empty cardboard boxes and plastic bags will be disposed of within an approved waste disposal facility. Unused bait pellets will be disposed of as hazardous waste according to U.S. Government regulations.

(a) (9) Additional information

This pesticide formulation is the same as Ramik Green (EPA Registration No. 2393-498) except it is formulated in a 6-gram pellet.

(a) (10) Supporting documentation

Attachment G. Letter of support from the National Park Service's Hawaii Volcanoes National Park for this research.

Attachment H. Letter of support from the Nature Conservancy of Hawaii for this research.

Attachment I. Letter of support from the U. S. Fish & Wildlife Service's Hakalau Forest National Wildlife Refuge for this research

Attachment J. Letter of support from the State of Hawaii, Department of Fish and Wildlife for this research.

2. Tolerance Requirements

We request an exemption from the requirement of a tolerance. The proposed experimental use pesticide application will be applied only within a non-food crop forestry area for the control of rats (*Rattus* spp.). The treatment area is a mesic forest within Hawaii Volcanoes National Park. Vegetation within this mesic forest is composed of a tall koa, ohia and soapberry trees. The dominant ground cover consists of native ferns and herbs, but blackberry and alien grasses are found in some areas of the forest. The treatment area does not contain standing water impoundments or streams. The area has been free from feral ungulates since the mid-1980's.

3. Data Requirements

See enclosed authorization letter from HACCO, Inc. for EPA to access data in EPA files on diphacinone to support this application.

4. Environmental Risks

Eisemann (2000; Attachment K) conducted preliminary aquatic and human health risk assessments for aerial applications of rodenticide baits containing diphacinone in Hawaii forest ecosystems. No predicted risk is indicated to aquatic species when risk quotients (RQ's) were calculated by the EPA surface water model, Generic Expected Environmental Concentration Program (GENEEC). Data also show that there is little risk of children consuming a toxic dose of diphacinone from drinking surface or ground water

An environmental assessment for testing broadcast bait applications of the Rodenticide Ramik Green conducted by the National Park Service resulted in a "Finding of No Significant Impact" to the environment (Attachment L).

A Section 7 Consultation on Experimental Use of Diphacinone Pellets in Hawaii Volcanoes National Park (Biological Opinion Log 1-2-99-F-03; Attachment M) by the U.S. Fish and Wildlife Service, Honolulu, HI concluded that the study is not likely to jeopardize the continued existence of the Hawaiian Hawk. Additionally, they conclude that the rodent control program may be beneficial to other endangered species within the project area.

Lindsey and Mosher (1994: Attachment F) assessed the secondary hazard potential of diphacinone to non-target avian predators, particularly the Hawaiian hawk or Io and the Hawaiian owl or Pueo (*Asio flammeus*), within forested areas of Hawaii. Dead rats (kill trapped) were placed on the forest floor to determine scavengers taking them. Mammalian predators rapidly located (mean of 2.9 days) and consumed the dead rats. Avian predators did not locate or take any of the exposed, dead rats. Radio-marked rats moving 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 owls as a result of poisoning from diphacinone was considered minimal.

5. Labeling Requirements

A draft label addressing use conditions and application directions required by EPA for shipment of the 0.005% diphacinone baits under an Experimental Use Permit is attached (Attachment N).

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. 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.

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. 260pp.

Buckle, A. P. and M. G. P. Fenn. 1992. Rodent control in the conservation of endangered species. Pp. 36-51 *in* J. E. Borrecco and R. E. Marsh, eds. Proceedings, 15th Conf. Vertebrate Pest Council. Univ. of Calif at Davis.

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.

Dunlevy, P., E. Wm. Campbell III, and G. Lindsey. 2000. A simulation study of broadcast application of rodenticide bait in a native Hawaiian forest. International Biodeterioration and Biodegratation. IN PRESS.

Eisemann, John D. 2000. Preliminary aquatic residue modeling and risk assessment for aerial application of diphacinone baits in Hawaii forest ecosystems. Unpublished report. 8pp.

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

Godfrey, M. E. R. 1985. Non-target and secondary poisoning hazards of "second 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.

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

IPCS. 1995. Environmental Health Criteria 175: Anticoagulant rodenticides. World Health Organization, Geneva. 123pp.

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.

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

Lindsey, G. D. and C. Forbes. 2000. Effectiveness of hand broadcast baiting with 0.005% diphacinone bait pellets in reducing rat populations in Hawaiian forests. Unpublished Report. 23pp.

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

Miller, C. J. and S. Anderson. 1992. Impacts of aerial 1080 poisoning on the birds of Rangitoto Island, Hauriaki Gulf, New Zealand. New Zealand J. Ecol. 16:103-107.

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.

Moors, P. J., I. A. E. Atkinson, and G. H. Sherley. 1992. Reducing the rat threat to island birds. Bird Conser. Intnl. 2:93-114.

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.

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.

Seto, N. W. H. and S. Conant. 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.

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. 431pp.

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.

Stone, C. P. 1985. Alien animals in Hawaii's native ecosystems: toward controlling the adverse effects of introduced vertebrates. Pp. 251-297 *in* C. P. Stone and J. M. Scott, eds. Co-operative National Park Resources Studies Unit. Univ. Hawaii, Honolulu.

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 375pp.

Woodward, P. W. 1972. The natural history of Kure Atoll, Northwestern Hawaiian Islands. Atoll Res. Bull. 164. 318pp.

ATTACHMENTS

A. Map showing location of treatment area in Kipuka Ki, Hawaii Volcanoes National Park, Island of Hawaii.

B. 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.

C. Dunlevy, P., E. Wm. Campbell III, and G. Lindsey. 2000. A simulation study of broadcast application of rodenticide bait in a native Hawaiian forest. International Biodeterioration and Biodegratation. IN PRESS.

D. Lindsey, G. D. and C. Forbes. 2000. Effectiveness of hand broadcast baiting with 0.005% diphacinone bait pellets in reducing rat populations in Hawaiian forests. Unpublished Report. 23pp.

E. Toxicant Working Group Draft Meeting Minutes, August 26, 1999.

F. 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.

G. Letter of support from the National Park Service's Hawaii Volcanoes National Park for this research.

H. Letter of support from the Nature Conservancy of Hawaii for this research.

I. Letter of support from the U. S. Fish & Wildlife Service's Hakalau Forest National Wildlife Refuge for this research.

J. Letter of support from the State of Hawaii, Department of Fish and Wildlife for this research.

K. Eisemann, John D. 2000. Preliminary aquatic residue modeling and risk assessment for aerial application of diphacinone baits in Hawaii forest ecosystems. Unpublished report. 8pp.

L. Environmental compliance for a test broadcast of the rodenticide Ramik Green and Environmental Assessment: test broadcast rodenticide Ramik Green to control rats, Hawaii Volcanoes National Park, Hawaii, Sept. 3, 1999.

M. Section 7 consultation on Experimental use of diphacinone pellets in Hawaii Volcanoes National Park: Biological Opinion (Log Number 1-2-99-F-03).

N. Draft label: Ramik Green For Experimental Use Only.

Appendix 11. Amendments to protocols in EUP application.

Amendment No. 1. Item 1. (a) (4) Study area.
Amendment No. 2. Item 1. (a) (4) Live trapping.
Amendment No. 3. Item 1. (a) (4) Census blocks.
Amendment No. 4. Item 1. (a) (4) Addition of house mouse monitoring.
Amendment No. 5. Item 1. (a) (4) Bait disappearance rate.
Amendment No. 6. Item 1. (a) (4) Analytical chemistry.
Amendment No. 7. Item 1. (a) (4) Non-target hazards.
Amendment No. 8. Item 1. (a) (4) Amount of pesticide product proposed for use.
Amendment No. 9. Item 1. (a) (4) Mode of dispersal.
Amendment No. 10. Item 1. (a) (4) Treatment site.
Amendment No. 11. Item 1. (a) (6) Name and address of all cooperators.

Amendment No. 1

Item to be changed: 1. (a) (4) Study area. Two 45.3 ha (112 acres; 673 × 673 m) study plots will be established in a mesic forest at Kipuka Ki (treatment) and Kipuka Puaulu (control) within Hawaii Volcanoes National Park on the island of Hawaii, State of Hawaii.

Revision: Two 45.56 ha (112.58 acres; 675 × 675 m) study plots will be established in a mesic forest at Kipuka Ki (treatment) and Kipuka Puaulu (non-treatment) within Hawaii Volcanoes National Park on the island of Hawaii, State of Hawaii.

Reasons: The study plots were increased in size to 675 × 675 m for ease of measurement, and to have a 200 m buffer around the 275 × 275 m core monitoring area.

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

Study Director: Dan Foot Date 8/25/03

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

Amendment No. 2

Item to be changed: 1. (a) (4) Live trapping. Traps will also be opened 2 weeks before aerial baiting, and 2 weeks, 2 months, and 4 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.

Revision: Traps will be opened 2 weeks before aerial baiting, and 3 weeks, 3 months, and 6 months after aerial bait application to determine efficacy and rat reinvasion 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). The capture rates cannot be compared statistically because there was no replication of plots (only pseudo-replication of traps) and there was a different number of trap nights in each trapping session.

Reasons: In earlier studies with Ramik® Green bait hand broadcast over 10-acre study plots, reinvasion of rats occurred within 2 months. To determine the effect of aerial baiting over a larger study plot (112.6 acres), it was decided to monitor rat reinvasion rates at longer intervals of 3 and 6 months.

It was not possible to compare rat capture rates pre- and post-treatment in the control and treatment plots because there was no replication of plots (only pseudo-replication of traps). Chi-square analysis could not be used on the raw data (number of rats caught) because the analysis could not take into account the different number of trap nights in each trapping session, resulting from different numbers of target and non-target species caught, nor could it allow for spatial or temporal correlation between trappings.

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

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 3

Item to be changed: 1. (a) (4) Census blocks. One week before the treatment plot is baited, 132 non-toxic census bait blocks will be placed on a 25- x 25-m spacing within each 45.3-ha plot. Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5m, 62.5m, etc.) for 2 nights before baiting, and at 3 weeks, 9 weeks and 17 weeks following baiting to determine efficacy and rat re-invasion.... The proportion of bait blocks gnawed on by rats pre- and post-treatment in the control and treatment plots will be compared using Chi-square analysis.

Revision: Two weeks before the treatment plot is baited, 132 non-toxic census blocks will be placed on a 25- × 25-m spacing within the central 275 × 275 m of each 45.6 ha plot. Blocks will be placed on transects in between live trap locations (i.e., 12.5 m, 37.5m, 62.5m, etc.) for 2 nights before baiting, and at 3 weeks, 3 months, and 6 months following baiting to determine efficacy and rat re-invasion.... The proportion of bait blocks gnawed on by rats pre- and post-treatment in the control and treatment plots cannot be compared statistically because there was no replication of plots and there was a different number of bait blocks remaining to be monitored in the morning in each monitoring session.

Reasons: The timing of census bait block monitoring was altered to co-ordinate with other rat monitoring activities (e.g., live trapping).

It was not possible to compare the proportion of bait blocks gnawed on by rats pre- and post-treatment in the treatment and non-treatment plots for the same reasons as for live trapping (i.e., there was no replication of plots, only pseudo-replication of bait blocks, and there was a different number of bait blocks remaining in the morning in each monitoring session because different numbers disappeared from unknown causes overnight).

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

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 4

Item to be changed: 1. (a) (4) Addition of house mouse monitoring.

Revision: Kill trapping. Fifty-six mouse traps (Victor snap traps) will be located at 10-m intervals (two traps per location) along one transect line in each study plot to estimate house mouse densities pre- and post-treatment. The traps will not be placed in protective trapping tunnels. Trapping will be carried out 3 weeks before, and then 1 month, 3 months, and 6 months after bait application. Within each trapping session, the traps will be baited with coconut chunks, set, and examined daily for 2 days (maximum 112 trap nights). Captured mice will be placed in marked containers, frozen, and sent to Genesis Laboratories (Wellington, CO) or Landcare Research (Lincoln, New Zealand) for analysis of diphacinone residues. The traps will be removed after a trapping session, and relocated to a new transect for the next trapping session. Mouse capture-rates pre- and post-treatment in the treatment and non-treatment plots will be calculated following the method of Nelson and Clark (1973).

Reasons: Monitoring house mouse activity before and after bait application was added to increase our knowledge of the effect of bait application on species other than rats.

Effect of Amendment: This amendment will provide additional information that will potentially improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 5

Item to be changed: 1. (a) (4) Bait disappearance rate. Immediately after the first 11.25 kg/ha bait application, 20 individual bait pellets will be randomly located within the center 4 hectares of the 45.3 ha treatment plot.

Revision: Immediately after the first and second 11.25 kg/ha bait applications, 20 individual bait pellets will be randomly located within the first quarter of the central 275 × 275 m monitoring area of the 45.56-ha treatment plot.

Reasons: Monitoring bait disappearance after the second bait application was added to increase our knowledge of bait disappearance. Locating the baits in the first quarter of the central monitoring area was done to follow the standard operating procedure SOP BRD-12 (see Appendix 22). However, data on the size, softness, surface area gnawed by rats, mice, or invertebrates, and the surface area covered by mold were not recorded, as specified in SOP BRD 12, because they were found to be of little use in the earlier hand-broadcast trial.

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

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 6

Item to be changed: 1. (a) (4) Analytical chemistry. The concentration of diphacinone in the baits will be analyzed by Genesis Laboratories, Inc., Wellington, CO.

Revision: The concentration of diphacinone in the baits will be analyzed by Genesis Laboratories, Inc., Wellington, CO. The concentration of diphacinone in animal livers will be analyzed by Genesis Laboratories, Inc., Wellington, CO, or Landcare Research, Lincoln, New Zealand.

Reasons: Diphacinone residues were analyzed in animal livers as well as in baits. Both laboratories are GLP accredited. The choice of laboratory was determined by cost-effectiveness.

Effect of Amendment: This amendment will provide additional information that will potentially improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 7

Item to be changed: 1. (a) (4) Non-target hazards. Four randomly selected transects (673 m long × 5 m wide and spaced at least 50 m apart; 3% 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.

Revision: Four randomly selected transects (475 m long × 5 m wide; and spaced at least 25 m apart; 2% of each study plot) within the treatment and non-treatment plot will be walked to search for non-target mortality 2 weeks before, and 3 weeks, 3 months, and 6 months after bait distribution.... Birds of four introduced species, viz., Kalij Pheasant (*Lophura leucomelana*), Red-billed Leiothrix (*Leiothrix lutea*), Northern Cardinal (*Cardinalis cardinalis*), and Japanese White-eye (*Zosterops japonicus*), will be collected by shooting or mist-netting in the treatment plot 1 month after the first bait application. The birds will be frozen and stored for possible determination of diphacinone residues.... Invertebrates in contact with baits will be recorded from baits placed at 2.5 m intervals beside wire flags on four transect lines, of 25 baits per line, after the first and second bait applications. Observations will be made during the day and at night, using a headlamp. Invertebrates will be identified to species where possible, otherwise genus, family, order, or class. Samples will be collected, frozen, and stored for possible future analysis of diphacinone residues.

Reasons: The search transects were shortened to be consistent with the length of transect searched in the earlier hand-broadcast study. The timing of searching was altered to co-ordinate with rat monitoring activities (e.g., live trapping). The collection of birds for diphacinone analysis and observation of invertebrates on baits were added to provide additional information on non-target risks of bait application.

Effect of Amendment: This amendment either does not affect or improves the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 8

Item to be changed: 1. (a) (4) Amount of pesticide product proposed for use.
22.5 kg/ha (20 lbs/acre). 1019.25 kg (2240 lb) total.

Revision: 22.5 kg/ha (20 lbs/acre). 1025 kg (2252 lb) total.

Reasons: The increase in treatment area from 673 × 673 m to 675 × 675 m (45.3 ha to 45.56 ha) meant that more bait would be required in total. The application rate remains the same.

Effect of Amendment: This amendment does not affect the outcome of the study. The total amount of bait to be applied is still below the permitted amount (3000 lb).

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 9

Item to be changed: 1. (a) (4) Mode of dispersal. The mode of dispersal ... will be a 100-m swathe out of a Dryslinger II bucket slung under a Hughes 500 helicopter.

Revision: Baits will be dispersed out of a Lakeland Helicopters bait bucket slung under a Hughes 500 helicopter.

Reasons: The Lakeland Helicopters bucket is a specially designed for application of baits such as Ramik® Green.

Effect of Amendment: This amendment was made in an attempt to improve the quality of bait application, and consequently improve the outcome of the study.

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 10

Item to be changed: 1. (a) (4) Treatment site. The treatment site is a 45.3 ha (673 × 673 m) area of Kipuka Ki.

Revision: The treatment site is a 45.56-ha (675 × 675 m) area of Kipuka Ki.

Reasons: The size of the treatment site was increased slightly for ease of measurement and to have a 200-m buffer around the 275 × 275-m core monitoring area (see Amendment No. 1).

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

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Amendment No. 11

Item to be changed: 1. (a) (6) Name and address of all cooperators.

Thomas L. Hauptman
President, Pacific Helicopter Tours, Inc.
Kahului Heliport, Hanger 109
Kahului, HI 96732

Pacific Helicopters is certified in aerial applications of agricultural pesticides and will use a Hughes 500 to apply the bait.

Revision:

David Okida
Volcano Heli-Tours
PO Box 5595
Hilo, HI 96720

Volcano Heli-Tours is certified in aerial applications of agricultural pesticides and will use a Hughes 500 to apply the bait.

Reasons: Volcano Heli-Tours was a local company experienced with flying in Hawaii Volcanoes National Park, and was available at the time required.

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

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Appendix 12. Deviations from protocols in EUP application.

Deviation 1. 1. (a) (4) Radio telemetry.
Deviation 2. 1. (a) (4) Live-trapping.
Deviation 3. 1. (a) (4) Diphacinone baiting.
Deviation 4. 1. (a) (4) Amount of pesticide product proposed for use.
Deviation 5. 1. (a) (4) Environmental conditions.

Deviation No. 1

Protocol deviated: 1. (a) (4) Radio telemetry. Each rat will be monitored daily for two weeks following bait application to determine its fate.

Deviation: All rats were not monitored daily for two weeks following bait application.

Reasons: All rats died within 8 days of bait application, so they could not be monitored for two weeks.

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. 2

Protocol deviated: 1. (a) (4) Live-trapping. All rats captured will be identified to species, sexed, weighed, ear-tagged and released.

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

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

Effect of Deviation: This deviation meant that some information about the 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

Protocol deviated: 1. (a) (4) Diphacinone baiting. The 45.3-ha treatment plot will be aerial broadcast baited with the fish-flavored diphacinone bait... One half of the bait (11.25 kg/ha; 10 lb/acre) will be distributed aerially on Day 1 and the other half (11.25 kg/ha; 10 lb/acre) will be distributed on Day 5–7... The total amount of bait aerially distributed will be 22.5 kg/ha (20 lb/acre).

Deviation: Diphacinone bait was accidentally distributed outside the treatment plot, and broadcast at a higher rate than stated in the protocol on Day 1 and at a lower rate on Day 5.

Reasons: The gate in the bottom of the bait bucket jammed open at the end of the third flight over the treatment plot on Day 1 (25 October), resulting in bait being spread outside the treatment plot, though still within Kipuka Ki, as the helicopter turned around to fly back over the plot. The extra area covered was estimated, from finding bait on the ground, as 8.1 ha (20 acres). As a consequence, an additional flight had to be made, with additional bait, to ensure that the treatment plot was covered. It is estimated that, eventually, 539 kg of bait was applied over the treatment plot and 96 kg over the extra area, at a rate of 11.83 kg/ha (10.51 lb/acre), on 25 October. This rate of application was still higher than planned, as a consequence of the size of opening in the bottom of the bait bucket being too large (see Appendix 21). A smaller opening was used on Day 5 (30 October) in an attempt to reduce the rate of bait application. As a consequence, the rate of application was estimated as 10.56 kg/ha (9.39 lb/acre) on 30 October. Overall, the total rate of bait application was 22.39 kg/ha (19.90 lb/acre).

Effect of Deviation: This deviation was the result of a minor technical problem and inexperience. It would not normally happen. It did not affect the outcome of the study. The total rate of bait application (22.39 kg/ha) was slightly less than planned (22.5 kg/ha).

Study Director: _____ Date 8/25/03

QA Officer: _____ Date 8/25/03

Deviation No. 4

Protocol deviated: 1. (a) (4) Amount of pesticide product proposed for use.
1019.25 kg (2240 lb) total, amended to 1025 kg (2252 lb) total
(see Appendix 11, Amendment No. 8).

Deviation: The total amount of bait used was 1116 kg (2461 lb), although only 1020 kg (2249 lb) was estimated to have been applied to the treatment plot.

Reasons: See Deviation No. 3.

Effect of Deviation: This deviation did not affect the outcome of the study. The total amount of bait used was below the permitted amount (3000 lb).

Study Director: _____ Date 8/21/03

QA Officer: _____ Date 8/25/03

Deviation No. 5

Protocol deviated: 1. (a) (4) Environmental conditions. Daily rainfall and temperature will be recorded by 10 a.m. each morning for 2 weeks after bait application.

Deviation: Rainfall was collected for only 9 days and temperature for only 5 days after the first bait application.

Reasons: The radio-collared rats were all dead by 9 days after bait application. The temperature records after 5 days have been lost.

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 13. 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 USGS-Biological Resources Division, Kilauea Field Station, Hawaii National Park, HI; and the National Park Service, U.S. Dept. of Interior, Hawaii Volcanoes National Park. This product will be used for research purposes only under State of Hawaii Department of Agriculture Experimental Use Permits.

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.

HACO, INC.•P.O. BOX 7190•MADISON, WI 53707
NET CONTENTS: 50 pounds (22.68 Kg.)
CODE L/00

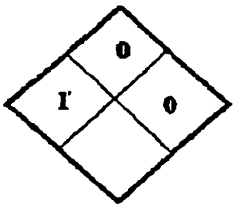
Appendix 14. Material safety data sheet.

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 15. HACCO certificates of analysis for 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: September 2000
Date Analyzed: September 2000

LOT NUMBER	% DIPHACINONE
144548	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: May 2002

LOT NUMBER	% DIPHACINONE
144548	0.0050

Jodi Fields
Quality Control Lab Technician

9-9-02
Date

MaryAnn Douglas
Research Chemist

9/9/07
Date

Appendix 16. End use product tracking form for Ramik® Green bait.

DATE	DESCRIPTION	QUANTITY OUT (lb)	QUANTITY REMAIN (lb)	INITIALS
16 Apr 2001	Received 52 x 50lb boxes from HACCO (total 2600 lb), stored in locked, dehumidified room, Bldg 216, BRD		2600	CFP
19 Apr 2001	Removed 60 pellets for measurement, then returned them			DF
30 Apr 2001	Sent 536 g to Genesis Labs via Fedex for analysis	1.2	2598.8	CFP
8 May 2001	Sent 71 pellets to HACCO for size measurement (ca. 426 g)	0.9	2597.9	IS
25 Oct 2001	Used 635 kg for aerial application in Kipuka Ki	1399.9	1198	CFP
30 Oct 2001	Used 481 kg for aerial application in Kipuka Ki	1060.4	137.6	CFP
30 Jan 2002	Sent 482 g to Genesis Labs via Fedex for analysis	1.1	136.5	CFP
26 Apr 2002	Gave 5100 g to Peter Dunlevy, APHIS, for slug & snail study	11.24	125.3	CFP
30 Apr 2002	Sent 120.5 g to HACCO via Fedex for analysis	0.27	125	CFP
18 Jun 2002	Used 100 lb, hand broadcast, Ola'a Forest under State EUP	125	0	CFP

CFP is Charlotte Forbes Perry
DF is David Foote
IS is Ilana Stout

Appendix 17. 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.

TABLE OF CONTENTS

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

Page 1 of 8

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

TABLE OF CONTENTS

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 — Date: 3/24/00

Genesis Management — Date: 3/24/00

Gerald O Lindsey
Sponsor/Sponsor Representative — Date: 4/4/00

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

① Study Director
~~Sponsor/Sponsor Representative~~ 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 7/17/02
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

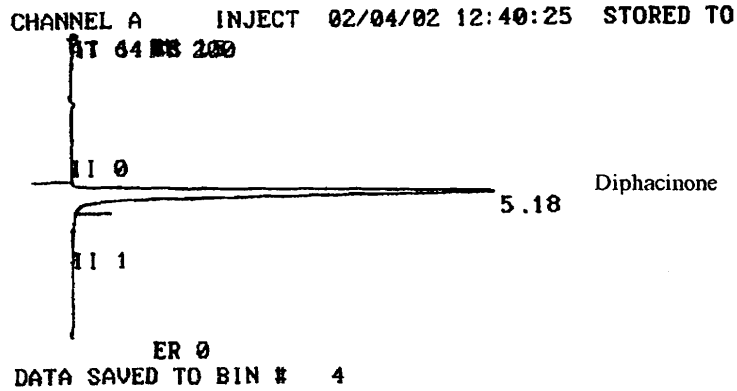
[1] - according to the chain of custody forms; [2] - two replicate injections of the same sample were done

APPENDIX III

REPRESENTATIVE CHROMATOGRAMS

Appendix III-a

Representative Chromatogram of Diphacinone Analytical Standard for the Bait Analysis
(11.8 µg/mL)



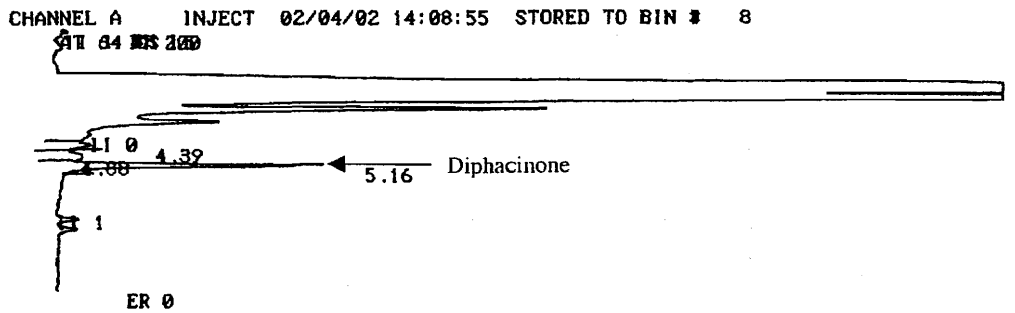
00005-BAIT/VM 02/04/02 12:40:25 CH= "A" PS= 1.

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



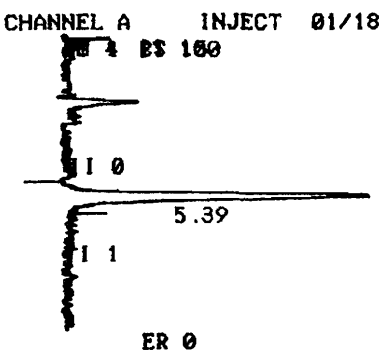
00005-BAIT/VM 02/04/02 14:08:55 CH= "A" PS= 1.

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

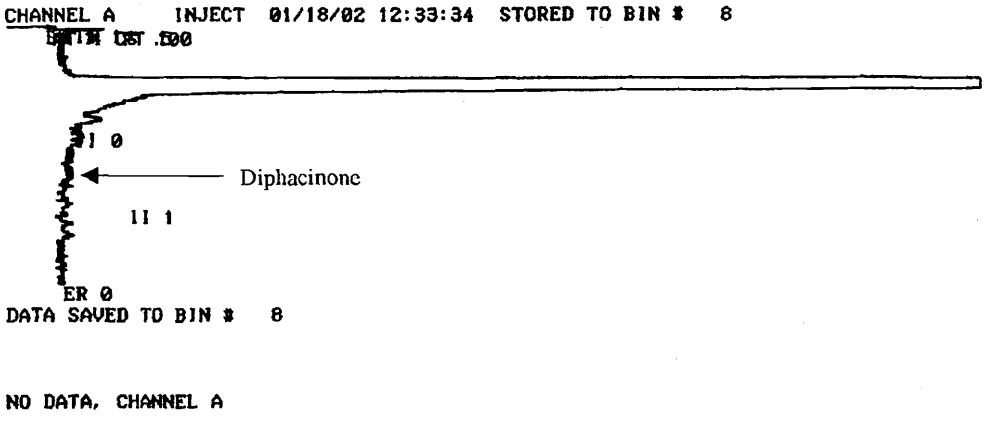
00005-LIVER/VM 01/18/02 11:40:17 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 4 INDEX 4 BIN 4

PEAK#	AREA%	RT	AREA	BC
1	100.	5.39	33626	41
TOTAL	100.		33626	

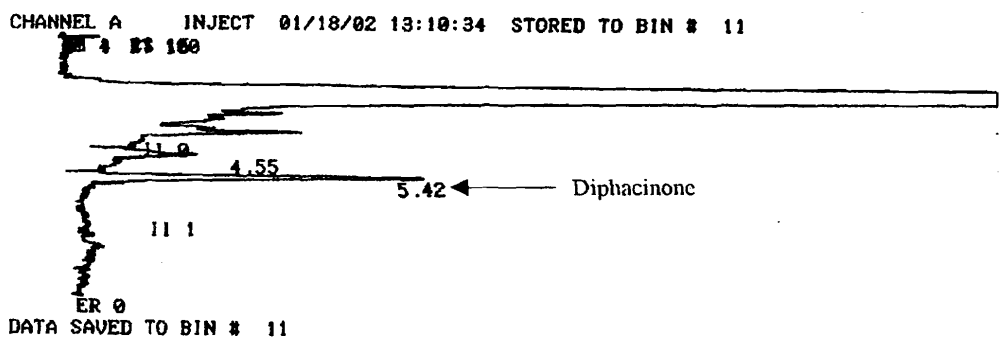
Appendix III-d

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 18. Flow rate of Ramik® Green bait through the helicopter bait bucket.

Objective

To determine the appropriate size of opening in the bottom of the helicopter bait bucket to deliver the required flow rate of Ramik® Green baits.

Background

The required flow rate of baits (kg/s) through the helicopter bait bucket was estimated from the required application rate of baits on the ground (11.25 kg/ha), the helicopter speed (30.833 m/s), and the swathe width of baits (96.4 m) applied by the helicopter bait bucket (see Appendix 19).

Required flow rate $= 11.25 \text{ kg/ha} \times 30.833 \text{ m/s} \times 96.4 \text{ m} / 10{,}000 \text{ m}^2$
$= 3.34 \text{ kg/s}.$

The size of opening in the bottom of the helicopter bait bucket required to deliver this flow rate was determined from a flow rate trial.

Methods

The bait bucket was suspended above the ground and loaded with 68 kg of Ramik® Green bait. The gate in the bottom of the bucket was then opened, the agitator started, and the time taken for the bait to pass out of the bucket was recorded. This was repeated with different sized openings, created by inserting different sized plastic and wooden rings in the bottom of the bucket. Only five plastic rings (90, 94, 98, 102, and 106 mm internal diameter) were supplied with the bucket. These were tested first. When these proved too small, additional rings were made, initially temporary wooden rings (120, 124, 126, 128, 130, and 143 mm) and then more permanent plastic rings (116, 120, 124, and 143 mm), and these were then tested.

Results

The bait flow rate was linearly related to the size of opening in the bottom of the bait bucket (Fig. A18).

Conclusions

Based on the linear regression, the plastic ring size that gave the flow rate closest to the desired flow rate was 125 mm internal diameter (3.35 kg/s). However, this size ring was not available. The closest plastic ring available was 124 mm internal diameter (3.27 kg/s), equivalent to 11 kg/ha, slightly less than wanted. This ring was available only for the second toxic bait application.

Based on the linear regression, the wooden ring size that gave the flow rate closest to the desired flow rate was 130 mm internal diameter (3.34 kg/s). However, the 130 mm ring tested had a flow rate of 3.62 kg/s. Consequently, the 128 mm wooden ring (which had a flow rate of 3.56 kg/s) was recommended for the first toxic bait application because, of the rings available, its flow rate was closest to the desired flow rate. The flow rate of baits was slightly slower through wooden rings than through plastic rings of the same size, probably because the wooden rings had square, rough edges whereas the plastic rings had rounded, smooth edges.

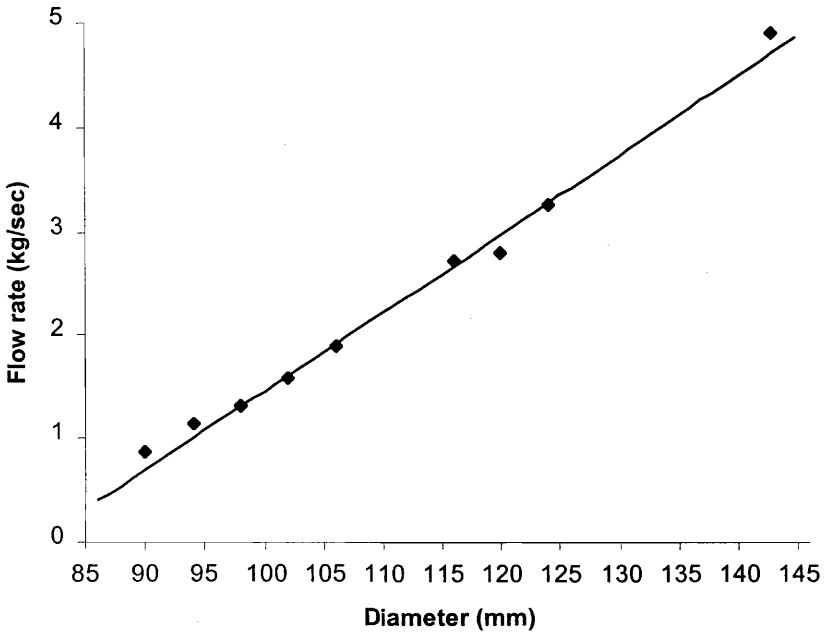


Fig. A18 (a). Flow rate of baits through plastic rings of different internal diameter (with linear regression line fitted through data points)

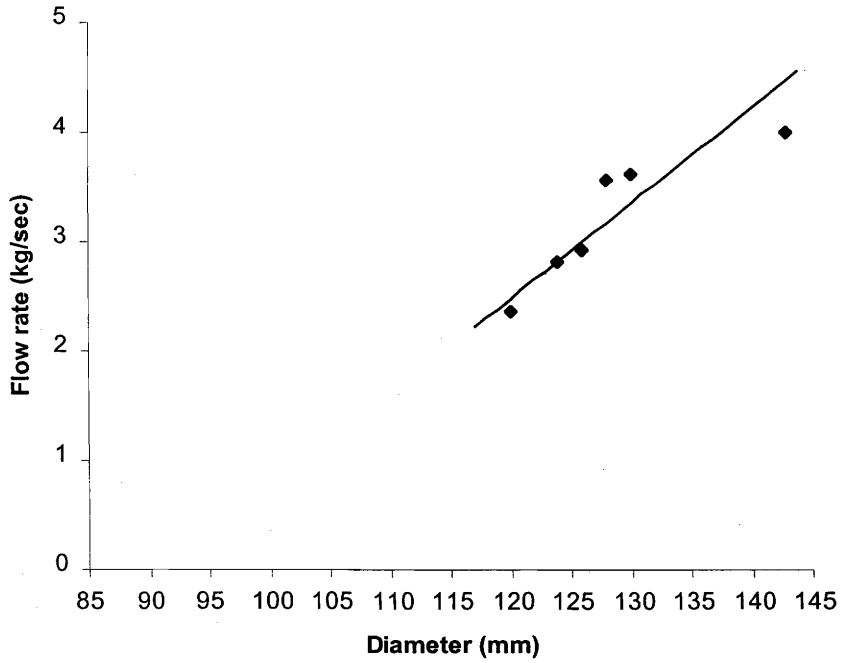


Fig. A18 (b). Flow rate of baits through wooden rings of different internal diameter (with linear regression line fitted through data points)

Appendix 19. Swathe width of Ramik® Green bait applied by the helicopter bait bucket.

Objective
To determine the swathe width of Ramik® Green bait applied by the helicopter bait bucket.

Background
It was necessary to determine the swathe width of Ramik® Green bait applied by the helicopter bait bucket to be able to calculate the number of helicopter flights (swathes of bait) required to cover the treatment area.

Methods
The swathe width was determined from a trial aerial application of placebo Ramik® Green bait over short pasture on Keauhou Ranch, at a similar altitude to Kipuka Ki, on 20 October 2001. The placebo bait was supposed to be the same flavor, color, size, and formulation as the toxic bait, but without diphacinone. The weight, length, and width of a random sample of 60 placebo and 60 toxic baits was measured before application. The short pasture meant that there was a good chance that all baits would be found after application. The size distribution of placebo baits collected after application was compared to that before application (Appendix 20).

The bait was applied from a Lakeland Helicopters bait bucket slung under a Hughes 500D helicopter. The plastic rings supplied with the bucket were considered too small, so a wooden ring was made and inserted into the socket in the bottom of the bait bucket to provide an opening of 143 mm diameter. The helicopter applied a single swathe of baits approximately 500 m long. The swathe width was determined as follows. The outermost baits along the sides of the swathe were marked with colored wire flags for the full length of the swathe. Two parallel lines of hip-chain were then run along the two sides of the swathe so that all baits were within the boundary. The distance between these two lines was the maximum swathe width. Three randomly located 10-m-wide strip transects were marked at right angles across the swathe. Seven people walked side by side along each strip transect counting and collecting all baits (including pieces of baits) in 2.5-m intervals. The number and weight of baits per ha for each 2.5-m interval (25 m^2) was calculated. Thus, the swathe width that delivered bait at 11.25 kg/ha could be calculated to the nearest 2.5 m.

Results
The maximum swathe width was 117.5 m, but the bait density across the swathe varied (Fig. A19). No baits were found in the three outermost 2.5 m sectors on one side of all three randomly selected transects, so the swathe width for these transects was only 110 m. Even then, the bait density in the outer sectors of these transects was well below the 11.25 kg/ha required. Because of the sharp drop off in bait density at the edges of the swathe, the effective swathe width was estimated to be between 95 and 97.5 m.

The mean weight of bait collected across the swathe was 1.18 kg, equivalent to a bait application rate of 10.0 kg/ha for a 117.5-m-wide swathe, and 11.20 kg/ha for a 110-m-wide swathe. The bait application rate was calculated as 12.41 kg/ha for a 97.5-m-wide swathe, and 12.56 kg/ha for a 95-m-wide swathe. The placebo baits were significantly lighter (by more than 1 g), shorter, and fatter than the toxic baits (Table A19).

Table A19. Mean (and standard error) of the weights, lengths, and diameters of Ramik® Green and placebo baits used in the aerial broadcast trials (with degrees of freedom, *F* and *P* values from an analysis of variance).

Bait type	n	Weight (g) Mean (SE)	Length (mm) Mean (SE)	Diameter (mm) Mean (SE)
Ramik Green®	60	6.29 (0.06)	23.0 (0.24)	20.5 (0.19)
Placebo	60	5.11 (0.07)	19.9 (0.31)	21.6 (0.11)
Degrees of freedom		1,118	1,82	1,82
F value		142.5	56.1	11.9
P value		<0.001	<0.001	0.001

Conclusions

The results indicated that seven helicopter flights would be required to cover the 675-m-wide treatment area. With this number of flights, the average swathe width would be 96.4 m (675 m / 7 flights = 96.4 m), within the effective range of 95–97.5 m. This means there would be some overlap of baits applied on each swathe, but this should not matter because the bait density at the edges of each swathe was lower than required.

The mean application rate of approximately 12.5 kg/ha estimated for a 96.4-m swathe is slightly higher than the 11.25 kg/ha required. Thus, the size of opening in the bottom of the bait bucket should be less than 143 mm diameter used in this trial. The effect of the size difference between placebo and Ramik® Green baits on swathe width and application rate is unknown, but likely to be minor.

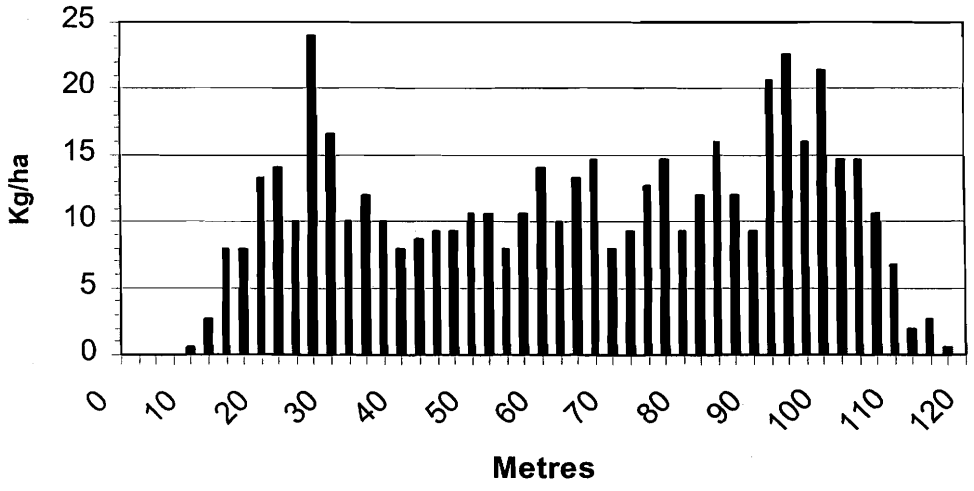


Fig. A19. Distribution of baits in 2.5-m intervals across the swathe from the helicopter bait bucket.

Appendix 20. Bait size before and after application by the helicopter bait bucket.

Objective

To determine the size distribution of placebo Ramik® Green baits before and after application by the helicopter bait bucket.

Background

The size distribution of placebo Ramik® Green baits was measured before and after application to determine whether there was any significant bait breakage as a result of being applied from the helicopter bait bucket.

Methods

The average weight of 686 placebo Ramik® Green baits collected from three random transects across a swathe of baits after bait application (Appendix 19) was compared with the average weight of 570 placebo Ramik® Green baits collected randomly from the boxes of baits before application. The comparison was restricted to baits weighing more than 1 g.

Results

The mean weight of individual placebo Ramik® Green baits more than 1 g was significantly lighter after the bait had been applied through the helicopter bait bucket than before (Table A20). Some baits were found that had been broken upon impact with rocks or logs on the ground. However, although there appeared to be more baits in the 4.0–4.9 g size class and fewer in the 5.0–5.9 g size class after bait application than before bait application, perhaps as a result of breakage (Fig. A20), the difference was not statistically significant (F = 1.124, df = 6,28, P = 0.374).

Table A20. Mean (and standard error) of the weights of placebo baits* before and after application through the helicopter bait bucket used in the aerial broadcast trial, Hawaii Volcanoes National Park, October 2001.

Bait type	Weight (g)	
	n	Mean (SE)
Before application	570	5.16 (0.02)
After application	686	4.93 (0.03)
F value		33.8
Degrees of freedom		1,1254
P value		<0.001

*Restricted to baits weighing >1 g (baits weighing <1 g comprised 0.7% of the weight before and 0.8% of the weight after application).

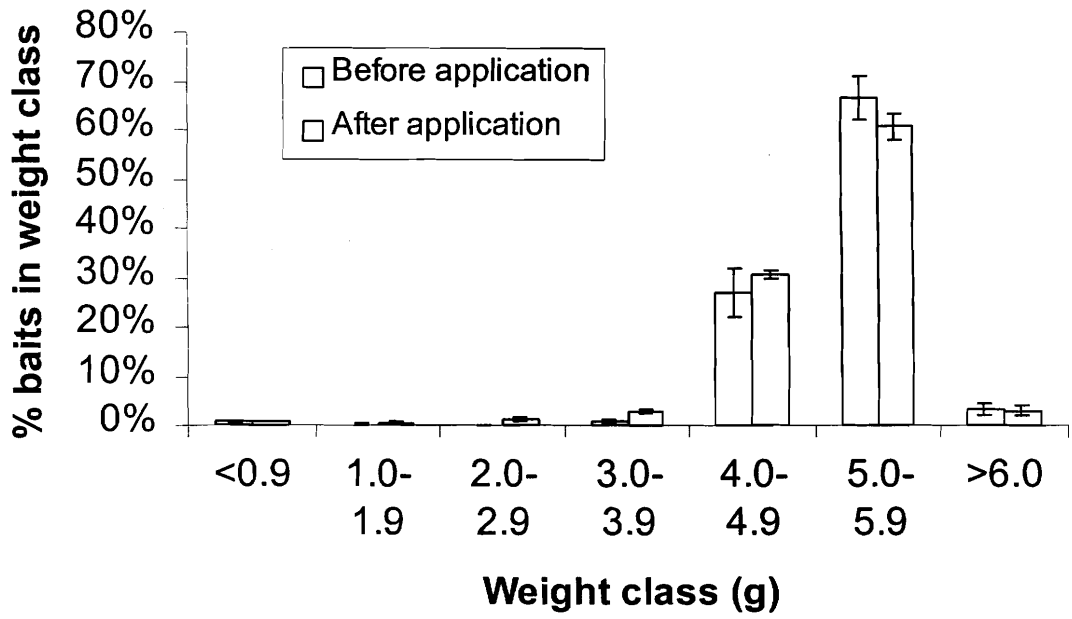


Fig. A20. Percentage (and standard error) of placebo baits in different weight classes before and after application through the helicopter bait bucket.

Conclusions

Aerial application of baits resulted in some baits being broken upon impact with the ground, but this did not have a significant impact upon the size distribution of baits.

Appendix 21. Accuracy of aerial application of toxic bait in the treatment plot.

Objective

To determine the accuracy of aerial application of toxic bait in the treatment plot.

Background

The protocol stated that the rate of bait application in the 45.56-ha treatment plot would be 22.5 kg/ha. This implies that (a) the total amount of bait applied would be 1025 kg (i.e., 45.56 ha × 22.5 kg/ha), (b) the bait would be applied to the treatment plot (not outside it), and (c) the bait would be evenly spread within the treatment plot.

Methods

Amount of bait. The amount of bait applied to the treatment plot was determined by recording the weight of bait loaded into the helicopter bait bucket and the weight of bait left after bait application.

Area covered by bait. Observers, carrying a compass and hand-help GPS (Garmin), walked along the outside edges of the treatment plot at the end of the helicopter flight paths on 25 October after the first bait application and again on 30 October after the second bait application, and searched for baits outside the treatment plot. The area covered by any bait found outside the treatment plot was mapped.

Distribution of bait within the treatment plot. Observers walked along the 12 transect lines in the central monitoring area of the treatment plot on 25 October after the first bait application and again on 30 October after the second bait application, searching for baits 1.5 m either side of the transect lines. Thus, the transects were strips 275 m long by 3 m wide (825 m^2). The number of baits found in 2.5-m intervals along the transects were recorded. There were 110 such plots on each transect, for a total of 1320 plots (measuring 2.5 m × 3 m), but 9 plots on 25 October and 10 plots on 30 October were not searched because of the dense growth of blackberry.

Results

Amount of bait. A total of 635 kg (1400 lb) of bait was applied on 25 October, and 481 kg (1061 lb) on 30 October, for a grand total of 1116 kg (2461 lb) (Appendix 16). Not all of the bait applied on 25 October was applied to the treatment plot (see Appendix 12, Deviation No. 3). The rate of application within the treatment plot was estimated as 11.83 kg/ha (10.51 lb/acre) on 25 October, and 10.56 kg/ha (9.39 lb/acre) on 30 October, for a total of 22.39 kg/ha (19.90 lb/acre).

Area covered by bait. Baits were found at the end of the helicopter flight paths up to 30 m outside the treatment plot on 25 October, and up to 80 m outside the treatment plot on 30 October.

Distribution of bait within the treatment plot. Baits were found on 45.4% of the 1320 plots (2.5 m × 3.0 m) in the treatment plot searched on 25 October after the first bait application (Fig. A21a). The average bait density within these plots was 858 baits/ha, which is 45.8% of the expected 1875 baits/ha.

Baits were found on 37.7% of the plots searched on 30 October after the second bait application (Fig. A21b). The average bait density was 695 baits/ha, which is 37.1% of the expected 1875 baits/ha. In addition, 746 baits/ha, or 87% of the baits from the first bait application were found. (In contrast, only 40% of 20 baits monitored for bait disappearance were found on 30 October – see Results, Section C). Baits from the first application were easier to find than baits from the second application because they had swollen (from absorption of moisture), and were therefore larger, and they had changed to a brighter green color, which contrasted more with the background vegetation.

Conclusions

The total amount of bait applied (1116 kg or 2461 lb) was more than planned (1025 kg or 2252 lb), but still below the permitted level (1361 kg or 3000lb). The reason for the greater amount of bait being applied on 25 October was because some bait was applied outside the treatment plot, though still within Kipuka Ki (see Appendix 12, Deviation No. 3).

The finding of baits outside the treatment plot at the end of the helicopter flight paths illustrates the difficulty that the pilot faces deciding when to close the gate in the bottom of the bait bucket. If the gate is closed when the helicopter is directly over the boundary, it is likely that bait will be spread outside the boundary as a result of forward momentum. The only solution is to stop applying bait before the helicopter reaches the boundary, leaving a strip around the inside of the boundary without bait, and then to apply bait to that strip by flying parallel to the boundary using differential GPS.

There were no large gaps in the distribution of baits found within the treatment plot (i.e., the bait distribution was relatively even). The percentage of the area covered by baits was less than 50%, but this is likely to be underestimated because less than 50% of the baits were found. The ability to find baits was affected by the density of the ground vegetation.

Fig. A21a. Distribution of baits found in the treatment plot on 25 October 2001, after the first aerial bait application (number of baits per 2.5 m × 3.0 m area).

Distance (m)	Transect A	B	C	D	E	F	G	H	I	J	K	L
2.5	1	0	0	0	1	0	0	1	1	0	1	1
5	0	0	0	3	1	1	0	0	0	0	0	0
7.5	1	0	0	0	0	0	0	0	1	1	0	1
10	0	0	0	0	0	1	0	0	0	0	0	1
12.5	0	0	0	1	0	0	0	0	1	0	0	2
15	0	0	0	0	1	0	0	0	1	1	0	1
17.5	0	0	0	2	1	0	0	0	0	0	0	0
20	1	0	0	0	1	0	1	0	0	0	0	1
22.5	0	0	0	0	0	0	1	0	1	0	0	1
25	0	0	0	0	0	0	0	0	0	0	1	0
27.5	1	0	1	1	0	0	1	0	1	0	0	0
30	1	1	1	0	0	0	0	0	0	0	1	0
32.5	0	0	0	0	0	1	0	1	0	1	0	0
35	2	0	0	0	0	0	0	0	0	1	0	0
37.5	1	2	1	1	1	0	1	0	0	0	2	1
40	1	1	1	0	0	0	0	0	0	1	1	1
42.5	0	0	0	1	0	0	1	0	1	0	1	0
45	0	1	2	1	0	0	1	0	0	1	1	0
47.5	1	0	0	0	1	0	0	0	2	1	1	2
50	2	0	0	1	1	0	2	1	1	0	2	0
52.5	0	1	0	0	0	0	0	0	0	0	3	0
55	0	0	0	0	0	0	1	1	0	0	0	0
57.5	0	0	0	0	0	0	1	0	0	0	1	2
60	0	0	0	0	1	0	1	1	1	0	1	0
62.5	0	0	1	0	2	2	1	0	0	1	1	0
65	0	0	0	0	1	0	0	0	0	1	2	2
67.5	0	0	0	0	1	0	0	0		0	2	1
70	0	0	0	0	1	0	2	0		0	3	1
72.5	1	0	0	0	1	0	1	0		1	2	2
75	0	0	0	0	0	0	0	0	0	1	2	0
77.5	0	0	1	0	0	0	0	1	1	1	1	1
80	1	1	0	0	1	0	1	1	2	0	0	0
82.5	1	0	0	0	2	0	0	0	0	2	1	0
85	0	0	0	0	2	0	0	0	1	0	0	0
87.5	2	2	0	0	0	0	0	1	1	0	1	1
90	4	2	0	0	0	0	0	1	0	0	1	0
92.5	1	0	0	0	0	0	0	1		1	0	1
95	2	0	0	6	1	1	0	1		1	0	0
97.5	1	1	0	0	0	2	0	0		0	0	1
100	1	0	0	1	1	0	0	0	0	0	0	0
102.5	0	2	0	1	0	0	1	0	2	0	0	1
105	1	0	0	1	0	0	2	0		0	0	1
107.5	0	0	0	0	0	0	0	1		0	0	1
110	0	0	2	0	0	0	1	1		0	0	1
112.5	1	0	0	1	0	0	0	1	0	0	0	0
115	2	0	1	1	0	0	1	0	0	0	0	3
117.5	0	0	0	0	0	0	0	1	0	0	0	1
120	0	0	0	0	0	1	1	0	0	0	0	1
122.5	2	0	0	1	0	0	1	0	0	0	1	0
125	1	0	0	0	0	2	2	0	0	0	0	0
127.5	0	0	0	0	0	0	1	0	0	1	3	0
130	0	0	1	1	0	0	0	0	0	0	1	1
132.5	0	0	0	0	0	0	1	0	1	0	1	0
135	1	0	1	0	0	1	2	1	0	0	0	0
137.5	1	1	2	1	1	1	1	1	0	1	1	0

140	0	0	0	0	0	0	0	1	0	0	0	0
142.5	1	0	0	0	0	0	1	1	0	0	1	1
145	0	0	0	0	0	0	0	2	0	0	1	1
147.5	1	0	0	0	0	0	0	2	1	0	0	1
150	0	0	0	0	0	0	0	2	0	0	0	0
152.5	0	0	0	1	0	0	1	1	1	1	1	1
155	3	1	0	0	1	0	1	0	0	0	0	0
157.5	0	0	0	0	0	1	1	2	0	0	0	0
160	0	0	0	0	0	0	1	0	0	0	0	1
162.5	0	1	0	0	1	1	1	1	0	0	1	0
165	0	0	1	0	0	1	0	2	0	0	0	0
167.5	1	0	1	0	0	0	0	0	0	0	1	0
170	1	0	2	0	0	1	0	3	0	0	0	2
172.5	0	0	2	1	0	0	1	1	0	0	1	1
175	0	2	0	0	0	1	1	1	0	1	0	1
177.5	1	0	0	1	0	0	2	2	1	0	0	2
180	1	1	3	1	2	1	0	3	0	0	1	2
182.5	2	1	0	0	0	1	1	2	1	0	2	1
185	3	0	0	0	0	0	0	2	2	1	1	3
187.5	2	4	0	2	1	0	0	1	1	0	0	2
190	2	1	1	1	0	0	0	0	2	1	1	0
192.5	2	1	1	3	0	0	1	3	0	1	1	3
195	1	1	2	0	1	0	1	2	0	1	2	4
197.5	3	0	1	0	2	0	1	2	2	0	2	4
200	0	2	0	1	1	0	1	2	1	1	3	1
202.5	4	0	0	0	0	0	0	2	1	0	4	4
205	1	1	0	0	0	0	1	1	1	1	2	3
207.5	0	0	0	1	0	0	0	2	0	1	0	3
210	1	2	0	0	1	1	0	0	0	1	4	2
212.5	1	2	0	0	0	1	1	0	1	2	3	1
215	0	0	0	0	0	0	0	1	0	0	4	2
217.5	0	1	0	0	1	0	1	1	1	3	5	0
220	0	0	0	0	1	1	0	2	0	2	2	0
222.5	1	0	0	0	0	0	0	1	0	1	3	1
225	0	0	0	0	1	0	1	1	0	1	1	2
227.5	0	0	1	0	0	1	1	1	0	1	3	0
230	1	1	1	1	1	1	3	1	0	1	3	2
232.5	1	2	1	0	1	1	2	2	1	0	1	1
235	1	0	0	1	2	1	2	1	1	1	3	0
237.5	0	2	0	1	0	1	1	2	2	2	1	1
240	1	0	0	3	1	0	2	1	1	1	2	1
242.5	1	0	2	0	0	0	3	1	2	1	1	0
245	0	1	1	2	1	1	1	2	3	0	2	0
247.5	3	2	1	0	1	1	3	2	0	1	1	2
250	1	2	4	2	1	1	3	2	1	1	0	0
252.5	1	0	1	0	3	0	1	0	1	1	0	1
255	1	0	2	3	3	0	1	0	1	2	0	2
257.5	0	0	0	0	1	0	1	1	1	2	0	1
260	1	0	1	0	2	2	1	0	2	1	0	3
262.5	1	1	1	0	1	0	1	1	1	0	1	3
265	0	1	1	1	0	0	1	1	1	2	2	1
267.5	0	2	2	2	1	0	0	1	0	0	3	1
270	0	1	0	0	1	1	1	0	1	3	1	1
272.5	2	0	1	1	1	0	1	1	1	0	0	2
275	4	1	1	4	1	1	2	4	3	0	3	2

Blank spaces indicate plots not able to be inspected (because of dense blackberry).

Fig. A21b. Distribution of new baits found in the treatment plot on 30 October 2001, after the second aerial bait application (number of baits per 2.5 m × 3.0 m area).

Distance (m)	Transect A	B	C	D	E	F	G	H	I	J	K	L
2.5	3	0	2	1	4	1	0	0	0	0	1	1
5	3	0	0	0	1	0	0	0	2	0	2	1
7.5	1	1	0	0	3	1	1	0	0	0	0	1
10	4	0	0	0	3	0	0	0	0	0	0	1
12.5	2	0	1	0	4	0	0	0	1	0	1	0
15	0	0	0	0	1	1	0	0	0	0	0	1
17.5	1	0	1	1	0	1	0	1	0	0	0	1
20	1	0	0	1	2	1	0	0	0	0	0	0
22.5	1	0	0	0	0	1	0	1	0	1	0	0
25	1	1	0	0	0	0	0	0	0	0	0	0
27.5	1	0	2	2	0	0	0	1	0	0	0	1
30	0	0	0	1	0	0	0	2	0	1	1	0
32.5	0	0	0	0	2	0	0	0	1	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	1	1	0	0	0	0	1	0
40	0	0	0	1	1	0	0	0	1	0	0	0
42.5	0	0	0	1	0	0	0	0	0	0	1	1
45	0	0	0	1	0	1	0	0	0	0	0	0
47.5	1	1	0	0	0	0	0	1	0	0	1	0
50	0	0	0	1	0	1	0	0	0	0	0	0
52.5	0	0	0	0	3	0	0	0	0	0	1	1
55	0	0	0	0	1	0	1	0	0	0	0	0
57.5	0	0	0	0	0	0	0	1	0	0	1	0
60	1	0	0	0	0	0	1	0	1	0	0	0
62.5	1	0	0	0	0	0	1	1	0	0	3	0
65	0	0	0	0	0	1	0	0	0	0	2	1
67.5	0	0	0	0	0	1	0	0		0	1	1
70	0	0	0	0	0	1	0	0		0	0	1
72.5	0	1	0	0	0	1	0	1		0	2	1
75	1	0	0	1	1	3	1	1	0	0	2	1
77.5	1	0	2	1	0	1	0	1	3	0	3	0
80	1	0	0	0	0	1	3	0	2	1	1	1
82.5	0	0	0	0	3	1	1	0	0	0	1	1
85	1	0	0	2	0	1	0	0	2	0	1	2
87.5	1	1	1	2	0	1	1	2	0	0	0	0
90	1	0	3	2	6	2	1	1	0	0	0	0
92.5	1	1	1	5	3	2	1	1		2	2	0
95	0	1	0	1	4	2	1	1		0	0	0
97.5	1	0	1	2	4	2	0	0		0	2	2
100	1	0	1	2	1	1	0	1	0	0	0	0
102.5	0	1	0	2	2	0	0	0	0	1	0	0
105	0	0	0	0	0	0	0	0		0	0	0
107.5	0	0	0	0	2	0	0	1		0	2	0
110	0	0	0	1	0	0	0	1		0	0	1
112.5	1	0	0	0	0	0	1	0		0	0	0
115	1	0	0	0	0	0	1	0	0	0	0	0
117.5	1	0	0	1	0	0	1	0	0	1	0	1
120	0	0	1	0	0	0	0	1	0	0	0	0
122.5	1	1	2	0	0	0	0	1	0	0	1	0
125	0	0	0	0	1	0	0	0	0	0	0	0
127.5	0	0	0	0	0	0	0	2	0	2	1	0
130	0	0	0	0	0	0	0	1	0	0	0	0
132.5	0	0	0	1	1	1	0	1	0	0	0	0
135	0	0	0	0	0	1	0	0	0	0	0	0
137.5	0	0	0	0	2	0	1	1	1	2	1	0

140	1	0	0	0	0	0	0	2	0	1	0	0
142.5	0	0	0	0	1	1	0	1	0	0	1	0
145	0	0	0	0	1	0	0	1	0	0	1	0
147.5	0	0	0	0	0	1	0	1	1	0	0	0
150	0	0	1	0	0	0	0	1	0	0	0	1
152.5	0	0	0	0	0	0	0	1	0	0	0	1
155	1	0	0	0	0	0	0	0	1	0	1	0
157.5	0	0	0	0	0	0	1	0	0	1	0	0
160	1	0	0	0	1	0	1	0	1	0	2	1
162.5	0	0	0	0	2	0	1	0	0	2	2	3
165	0	0	1	1	1	0	1	0	1	0	0	2
167.5	0	0	0	0	0	0	1	0	1	1	0	0
170	0	0	0	1	0	0	0	0	0	1	1	1
172.5	0	0	0	1	0	0	1	1	2	1	2	1
175	1	0	0	0	0	0	0	0	0	1	2	1
177.5	1	0	1	0	0	0	1	0	3	0	2	3
180	0	0	0	1	3	2	0	0	0	0	1	1
182.5	1	0	1	0	0	3	0	0	0	2	1	3
185	1	0	0	0	0	1	2	0	0	1	1	5
187.5	0	0	0	0	0	1	0	0	0	0	0	4
190	0	0	1	0	0	1	0	1	0	1	0	0
192.5	0	0	0	1	1	2	1	0	2	0	1	0
195	0	0	0	0	0	0	1	1	0	2	0	1
197.5	1	0	0	0	0	1	1	0	1	0	2	1
200	0	0	0	1	1	0	1	0	0	0	0	1
202.5	0	0	0	0	0	2	0	0	2	1	1	1
205	0	0	1	0	0	2	0	1	0	0	0	1
207.5	2	1	2	0	1	0	0	1	1	0	0	1
210	1	0	0	0	0	1	0	0	0	1	0	0
212.5	1	1	0	0	0	0	0	0	0	2	0	0
215	0	0	0	2	0	2	2	1	1	1	0	0
217.5	0	0	0	1	1	0	0	1	0	1	0	0
220	0	2	0	0	0	2	0	0	0	0	0	0
222.5	0	1	0	0	0	1	0	0	0	0	1	0
225	0	0	0	2	1	0	0	0	0	0	0	0
227.5	1	0	0	0	1	1	1	0	1	0	0	0
230	0	0	0	0	1	0	0	0	0	0	0	0
232.5	0	0	0	0	2	0	0	0	1	0	0	0
235	0	1	1	1	2	0	0	0	0	0	0	0
237.5	1	0	0	0	1	1	0	0	0	0	1	1
240	2	1	2	1	3	0	0	0	0	1	0	0
242.5	1	1	0	0	1	0	1	0	0	0	0	1
245	0	0	0	0	1	1	0	1	1	0	0	1
247.5	0	1	0	1	0	0	0	1	1	0	1	0
250	1	0	1	0	1	2	1	1	0	0	0	0
252.5	2	0	0	1	0	0	1	0	0	0	1	2
255	0	0	0	1	1	2	1	0	0	0	1	0
257.5	0	1	1	0	1	2	0	1	2	0	0	1
260	1	0	0	1	2	3	1	1	0	1	0	2
262.5	0	2	0	3	2	6	1	1	0	2	1	4
265	0	1	0	1	2	2	2	0	2	1	1	0
267.5	0	1	1	0	5	2	1	1	0	0	0	1
270	1	0	0	0	5	0	2	2	2	1	2	2
272.5	1	1	0	2	2	0	3	1	1	1	3	1
275	1	1	1	3	1	2	2	1	2	1	2	1

Blank spaces indicate plots not able to be inspected (because of dense blackberry).

Appendix 22. Standard Operating Procedures (SOPs).

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 bait blocks in the field to estimate densities of rats (*Rattus* spp.).
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: 8/25/03

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

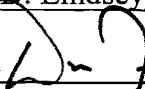
3.1 Nighttime radio-tracking

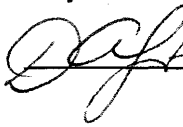
a). Walk into study area using headlamps.
b). Identify and locate signal of each radio-marked rat using 2-element antenna.
c). Listen to each signal without moving antenna or receiver for 4 minutes. If signal strength is fluctuating, then record animal as moving. If signal strength is steady, then record 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.)

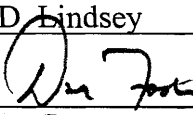
3.2 Ramik® Green

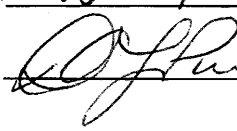
a). Read MSDS sheet for Ramik® Green
b). Wear long-sleeved shirt, long pants, boots and rubber gloves under cotton gloves to distribute bait pellets.

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 23. Timetable of monitoring activities.

Timetable of monitoring activities before and after aerial application of Ramik® Green bait, Hawaii Volcanoes National Park, 25 and 30 October 2001.

Stage	Dates	Activity
Before	2 Oct – 3 Oct	Snap-trapping mice
	11 Oct – 12 Oct	Census bait block monitoring
	15 Oct	Search for dead non-target species
	16 Oct – 19 Oct	Live-trapping
	22 Oct – 24 Oct	Radio-telemetry
After 1 month	26 Oct – 9 Nov	Radio-telemetry
	13 Nov	Search for dead non-target species
	13 Nov – 16 Nov	Live-trapping
	20 Nov – 21 Nov, 29 Nov – 30 Nov	Mist-netting and shooting birds
	29 Nov – 30 Nov	Snap-trapping mice
	5 Dec – 6 Dec	Census bait block monitoring
After 3 months	24 Jan – 25 Jan	Snap-trapping mice
	6 Feb – 7 Feb	Census bait block monitoring
	11 Feb	Search for dead non-target species
	12 Feb – 15 Feb	Live-trapping
After 6 months	25 Apr – 26 Apr	Snap-trapping mice
	2 May – 3 May	Census bait block monitoring
	7 May – 10 May	Live-trapping
	13 May	Search for dead non-target species

Appendix 24. Landcare Research 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

TOXICOLOGY LABORATORY ANALYSIS REPORT

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

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 05:14:35 p
Report Date: 6/20/02 11:57:07 a

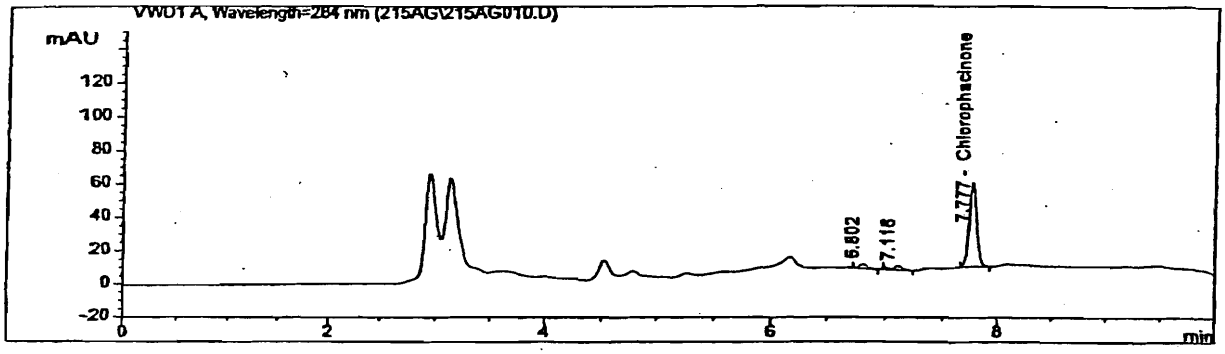
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.6

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

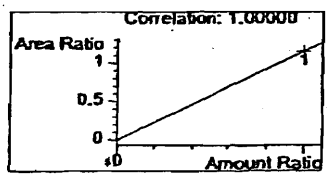
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

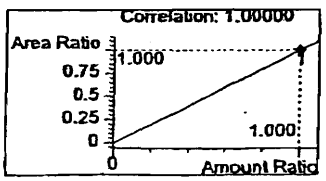
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 11:54:17 am



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 03:39:12 p
Report Date: 6/20/02 00:00:13 p

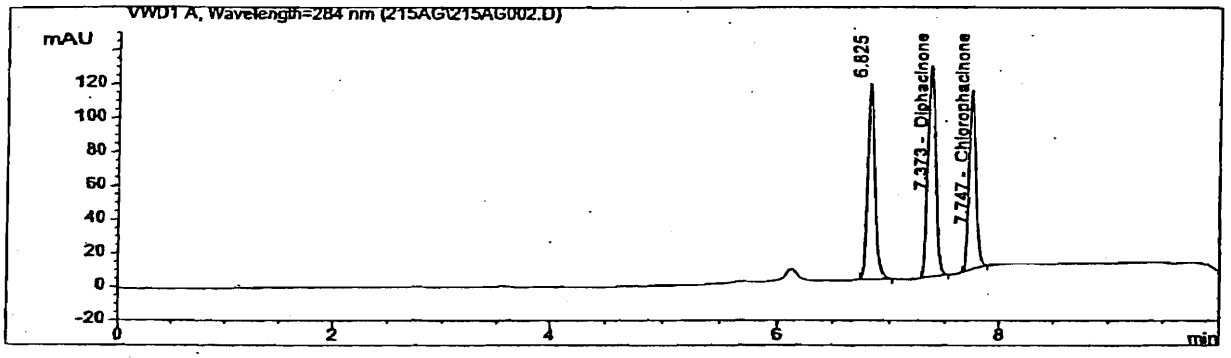
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.0

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

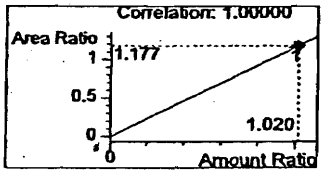
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

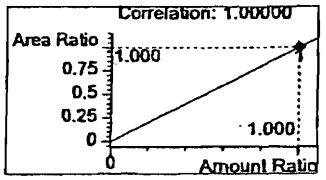
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 00:00:12 pm



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 05:26:30 p
Report Date: 6/20/02 11:57:11 a

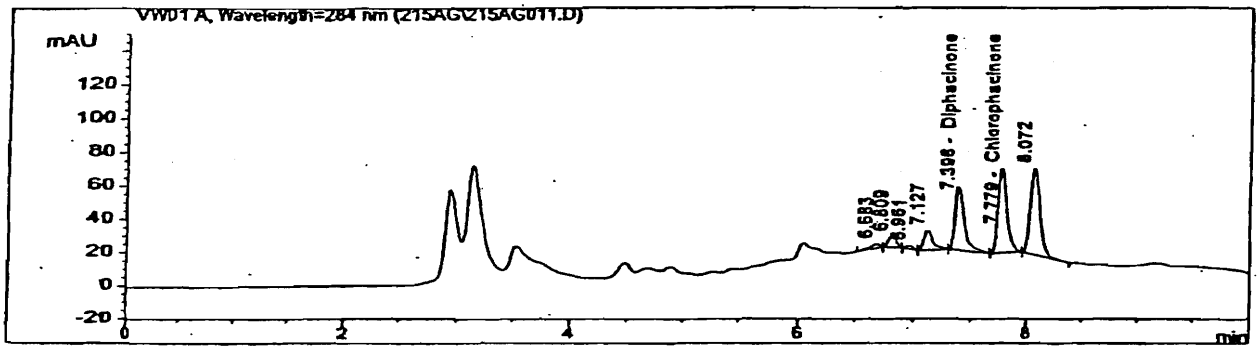
Injection Vol.: 100uL
Flow rate: 0.0
Start Pressure (bar): 8.5

Method:
C:\HPCHEM\1\METHODS\TOXLAB\DIPH_INT.M

Detector: UV
wavelength, nm : 280

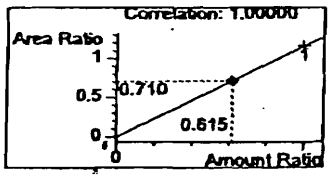
Solvent:
A: Methanol +0.005M TBAP
B: Water + PIC A

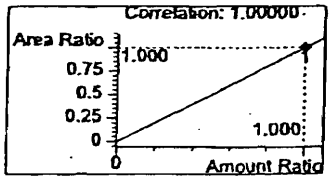
Calibration:
Calculated on :Area
Calibration created: 10/17/01
Calibration Modified:6/20/02 11:54:17 am



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





Appendix 25. Diphacinone residues in birds.

Birds were collected in the treatment plot ca. 1 month after aerial application of Ramik® Green bait, Kipuka Ki, Hawaii Volcanoes National Park, 25 and 30 October 2001 (see also Appendix 17 and 24).

Sample No.	Sample Description	Date Collected	Capture Method	Analysis Lab.	Lab. No.	Result (ppm)
B24	Kalij pheasant (male)	20 Nov 2001	Shot gun	Genesis	02-TS-02B	0.12
B25	Kalij pheasant (female)	20 Nov 2001	Shot gun	Genesis	02-TS-02A	0.18
B6	Red-billed Leiothrix	29 Nov 2001	Snap trap	Genesis	02-TS-02G	1.25
B8	Red-billed Leiothrix	29 Nov 2001	Pisonia tangle	Genesis	02-TS-02H	1.34
B9	Red-billed Leiothrix	29 Nov 2001	Snap trap	Landcare	4017	2.9
B10	Red-billed Leiothrix	29 Nov 2001	Snap trap	Landcare	4018	2.8
B12	Red-billed Leiothrix	29 Nov 2001	Snap trap	Landcare	4019	3.9
B14	Red-billed Leiothrix	30 Nov 2001	Snap trap	Landcare	4021	4.9
B15	Red-billed Leiothrix	30 Nov 2001	Mist net	Genesis	02-TS-02I	0.74
B17	Red-billed Leiothrix	30 Nov 2001	Snap trap	Landcare	4022	1.8
B11	Northern Cardinal	29 Nov 2001	Mist net	Genesis	02-TS-02J	0.13
B16	Northern Cardinal	30 Nov 2001	Mist net	Genesis	02-TS-02K	0.08
B3	Japanese White-eye	20 Nov 2001	Mist net	Genesis	02-TS-02D	0
B4	Japanese White-eye	21 Nov 2001	Mist net	Genesis	02-TS-02E	0
B5	Japanese White-eye	29 Nov 2001	Mist net	Genesis	02-TS-02F	0
B7	Japanese White-eye	29 Nov 2001	Mist net	Landcare	4016	0
B13	Japanese White-eye	29 Nov 2001	Mist net	Landcare	4020	0