Draft Supplemental Environmental Assessment
Lehua Island Ecosystem Restoration Project
July 2008

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Prepared in compliance with the National Environmental Policy Act and Hawai‘I HRS 343 and all associated regulations.

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Executive Summary

Lehua is an uninhabited island in Kaua‘i County, Hawai‘i located approximately 150 miles north-northwest of Honolulu, less than a mile north of Ni‘ihau, and approximately 20 miles west of the island of Kaua‘i. Its three-dimensional surface area is approximately 310 acres, although a variety of lower acreage figures are cited, likely based on estimates from 2-dimensional maps and images. The island is Federal property administered by the U.S. Coast Guard, which maintains a solar-powered navigational beacon near the 702-foot summit. It is also a state-designated Seabird Sanctuary managed by the Hawai‘i Department of Land and Natural Resources (DLNR), and is zoned as Conservation land.

The U.S. Fish and Wildlife Service and the Hawai‘i Department of Land and Natural Resources, Division of Forestry and Wildlife, in cooperation with the U.S. Coast Guard, propose to restore native species on Lehua Island by eradicating invasive rats using aerial application of bait pellets containing the anticoagulant rodenticide diphacinone (0.005% active ingredient). Bait with the anticoagulant brodifacoum (0.0025% active ingredient) would be considered for use if diphacinone failed to eradicate rats. The objective is to create suitable conditions for restoration of native seabirds, plants and other species by exposing all rats on Lehua to a lethal dose of rodenticide, thus eradicating rats from the island. The operation will be conducted during the winter months (December through February) when the rat population is low, few if any new rats are born, and native nontarget migratory species are not present or present in low numbers. Diphacinone has been shown to be an effective toxicant for rats in Hawaii and elsewhere and is preferred because of the reduced impacts to nontarget species, especially birds, both through consumption of bait (direct impacts) and/or through consumption of prey that has consumed the bait (secondary impacts).

In September 2005, the U.S. Fish and Wildlife Service and the Hawai‘i Department of Land and Natural Resources Division of Forestry and Wildlife, as joint lead agencies, and the U.S. Department of Homeland Security, U.S. Coast Guard, as the cooperating agency published the *Final Environmental Assessment for the Lehua Island Ecosystem Restoration Project*, (Finding of No Significant Impact (FONSI) dated 09/30/05). As documented in the FONSI, the U.S. Fish and Wildlife Service Assistant Regional Director, Ecological Services, Region 1 selected the proposed action, Alternative 2, which included the following:

1) Eradication of the introduced alien European rabbit (*Oryctolagus cuniculus*) and Polynesian rat (*Rattus exulans*) on Lehua Island, as these species prevent or suppress ecological regeneration, followed by implementation of a long-term ecological restoration strategy;

2) Adoption of a preventive strategy to reduce the potential for invasive species to be accidentally reintroduced to Lehua Island during and after restoration activities occur (island biosafety/quarantine strategy);

3) Reintroduce appropriate native species that cannot effectively recolonize on their own; and

4) Monitor project actions for effectiveness and overall restoration success.

Alternative 2 of the 2005 EA for Lehua included aerial and hand broadcast of bait pellets containing rodenticide in the summer months. The rodenticide proposed for use was diphacinone (50 ppm), with potential to use brodifacoum (25 ppm) as a backup the following year, but only if it could be determined that any eradication failure is due entirely to the diphacinone rodenticide and not other factors.

Following completion of the 2005 Final EA for ecological restoration of Lehua Island, European rabbits were eradicated from Lehua through intensive hunting efforts in 2005 and 2006. Therefore, rabbit eradication will not be addressed in this document.

Since the FONSI was signed in 2005, several important modifications to the rat eradication operation on Lehua Island associated with Alternative 2 have been determined to be more effective for rat eradication while also minimizing and/or avoiding adverse impacts to birds and humans. Therefore, the USFWS and DLNR, as joint lead agencies, have determined that the original 2005 EA should be supplemented to evaluate the impacts associated with these modifications. The purpose of this supplement is to describe the rat eradication operation for Lehua Island in detail as modified and evaluate the effectiveness and impacts associated with the entire operation, including the modifications.

The modifications include:

- Changing the season of starting rat eradication from mid-summer to mid-winter (December through February) in order to:
    - increase efficacy of the operation by exposing the rats to rodenticide during winter when breeding ceases or slows, the rat population is at a minimum, and there is a lower probability that young rats in underground burrows will not be exposed to rodenticide,
    - substantially decrease exposure of nontarget bird species to rodenticide since fewer birds are present in winter,
    - greatly reduce exposure of fishermen, limpet-pickers, and tourists, who rarely if ever use the area during winter,
    - reduce chances of helicopter bird strikes, since fewer seabirds will be present at that time, and
    - avoid all federally listed threatened and endangered seabird species, which are not present on Lehua during the winter.
- Improving effectiveness of bait distribution to all rats on Lehua by modifying or deleting those operational activities and mitigation actions that are not necessary to protect marine organisms, based on the extremely low risk and toxicity of bait to marine organisms as shown by the literature and by marine sampling results from the February 2008 Mokapu Island rat eradication near Moloka‘i. Specifically:
    - The deflector originally proposed for the bait applicator will not be used. Such deflectors, as currently designed, make it difficult for pilots to distribute bait pellets uniformly and frequently cause the bait applicator to malfunction;
    - To give the helicopter pilot and project manager discretion to distribute bait in the most effective pattern, the pilot will not be required to fly only from the coastline toward the ridgeline as originally proposed;
    - the project manager and pilot will not be excluded from applying bait adjacent to coastlines, thus ensuring an uniform and complete distribution of pellets in shoreline areas used by rats;
- If any broadcast of rodenticide pellets occurs after black-footed and/or Laysan albatross chicks hatch, then all pellets within 6 feet of the nest will be manually collected so that

chicks, which are not yet mobile, cannot play with or ingest them accidentally. All albatross nesting is localized near and at the top of the northwestern portion of the inner crescent, facilitating such removal.

This document also analyzes impacts of diphacinone and brodifacoum related to the modified operation, including:

- transport of rodenticides through soils and water
- impacts of rodenticides on terrestrial and marine invertebrates through ingestion
- impacts on nearshore fish from ingestion of rodenticide bait and ingestion of marine invertebrates potentially having rodenticide residues in their tissues
- impacts on human health
- impacts on birds present on Lehua in the winter, including certain species of native seabirds, nonnative passerine birds, the nonnative barn owl, and two native shorebirds.

The National Marine Fisheries Service determined that the modifications to the operation will not alter their original 2005 conclusion that the project "may affect but will not adversely affect" Hawaiian monk seals and sea turtles. The USFWS made the same determination regarding three rare species of seabirds observed on Lehua.

A Finding of No Significant Impact (FONSI) per NEPA is anticipated based on analysis in Chapter 3 and no significant impacts are anticipated per HRS 343.

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1.0 PURPOSE AND NEED

1.1 Description of Lehua Island and the Need for Rat Eradication

Lehua is an uninhabited island located approximately 150 miles north-northwest of Honolulu, less than a mile from Niʻihau, and approximately 20 miles west of the island of Kauaʻi. Its three-dimensional surface area is approximately 310 acres. Lehua is Federal property administered by the U.S. Coast Guard, which maintains a solar-powered navigational beacon near the 702-foot summit. It is also a state-designated Seabird Sanctuary managed by the Hawaiʻi Department of Land and Natural Resources (DLNR), and the land is zoned as a Conservation District. Ecological restoration of Lehua Island was identified as a goal in the USFWS Pacific Region Seabird Conservation Plan (USFWS 2005) and by the Offshore Islet Restoration Committee, which is a working group of Hawaiʻi conservation organizations and agencies. The Hawaiʻi State Comprehensive Wildlife Conservation Strategy (Mitchell 2005) identifies Lehua as one of two islands offshore of Kauaʻi (Kaula is the other) that is very important for seabird breeding.

An unidentified species of rat was first recorded on Lehua Island by Caum (1936), who reported that lighthouse personnel saw rats as early as 1931. Polynesian rats were positively identified during surveys conducted on Lehua in 2003 and 2004 (Wood et al. 2006) and voucher specimens were placed at Bishop Museum.

Polynesian rats are the smallest of the three alien rats introduced to Hawaiʻi. They eat a wide variety of foods, including fleshy fruit, seeds, flowers, stems, leaves, roots and other plant parts (Atkinson and Atkinson 2000). They also eat earthworms, centipedes, the larvae of butterflies and moths, ants, beetles, cicadas, snails and spiders. Rats scavenge and may also kill vertebrate prey, including birds and their eggs (Drummond 1960, Norman 1970, Fall et al. 1971, Jackson 1982, Atkinson 1985, King 1990, Navarette and Castilla 1993, Sugihara 1997, Drever and Harestad 1998, Hobsen et al. 1999, Cole et al. 2000, Innes 2001, Stapp 2002, Dunlevy and Scharf 2008). As reported in Tomich (1986), Polynesian rats in Hawaiʻi may prey upon Bulwer's petrel (*Bulweria bulwerii*), Laysan albatross (*Phoebastria immutabilis*) and burrow-nesting species such as the wedge-tailed shearwater (*Puffinus pacificus*), and the Bonin petrel (*Pterodroma hypoleuca*). Atkinson and Atkinson (2000) also reported detrimental effects of rats on burrowing petrels in Hawaiʻi and New Zealand and on red-tailed tropicbirds (*Phaethon lepturus*). Rat eradication on Midway Atoll resulted in dramatic increases of Bonin petrels, whose population had been declining due to rat predation (Seto and Conant 1996). In the two years immediately following the control of black rats on Mokoliʻi near Oʻahu, nesting success in wedge-tailed shearwaters increased rapidly, from only one chick fledging in the three years prior to rat eradication to 185 chicks fledging the second year after eradication (D. Smith, Hawai'i DOFAW, pers. comm.). Rats have also been documented to feed on endemic crickets and weevils (F. Howarth unpublished data, pers. comm.), as well as the seeds, bark, fruits, leaves and shoots of native Hawaiian plants.

Native seabirds, insects, coastal plants and marine species are becoming increasingly rare in the main Hawaiian Islands and have limited opportunities to recover due to alien species invasions, coastal development, and other human activities. Surveys conducted on Lehua Island in 1931 (Caum 1931) identified that European rabbits and Polynesian rats were the two main causes of native plant community degradation and the resulting dominance of nonnative plants there. Currently, about 23 native species, generally in very low numbers, have been able to survive

both rat and rabbit predation. Subsequent biological surveys have documented the extirpation or near extirpation of several species of native plants, insects, and seabirds by rats, rabbits, and other alien species, such as barn owls (*Tyto alba*) and cattle egrets (*Bulbucus ibis*) (Wood et al. 2004, VanderWerf et al. 2007). Guilds of native crickets, earwigs, mites, and spiders that were directly dependent on large numbers of breeding seabirds have disappeared from most islands due to eradication of large seabird colonies and the introduction of ants and other alien insects. Although rats have extirpated or diminished populations of several of the smaller, ground-nesting seabirds, Lehua still stands out as one of the largest and most diverse seabird colonies in the main Hawaiian Islands. Recent surveys documented over 25,000 breeding pairs of seabirds and up to 11 species nesting or attempting to nest on Lehua (VanderWerf et al. 2007).

Wedge-tailed shearwaters are the most numerous species on the island, but Lehua has the largest brown booby (*Sula leucogaster)* colony and one of the largest red-footed booby (*Sula sula*) colonies in Hawai‘i. Lehua and possibly Kaula are the only two nesting locations in the main Hawaiian Islands for rare black-footed albatross (*Phoebastria nigripes*), which were first documented nesting on Lehua in 2001 (Wood et al. 2004). Laysan albatross, another species rarely seen in the main Hawaiian Islands, also nest on Lehua. Another exciting discovery was the presence of rare band-rumped storm-petrels (*Oceanodroma castro*), threatened Newell's shearwaters (*Puffinus auricularis newelli*), and endangered Hawaiian petrels (*Pterodroma sandwichensis*) (VanderWerf et al. 2007). All three species have been seen returning to and circling Lehua in the evening. Biologists also found the body of a juvenile Newell's shearwater that was too young to fly, demonstrating that this rare and declining species is attempting to nest on Lehua but without much success. Species apparently extirpated from Lehua include the brown noddy (*Anous stolidus pileatus*), masked booby (*Sula dactylatra*), Bonin petrel, sooty tern (*Sterna fuscata*), gray-backed tern (*Sterna lunata)*, and blue-gray noddy (*Procelsterna cerulea*).

Once restored, Lehua Island can provide a safe haven for a diverse and abundant suite of coastal species. Despite its problems, including presence of alien rats (and formerly rabbits) since at least the 1930s, if not earlier, Lehua still supports a large seabird colony, including small numbers of very rare seabird species. Restoration of rare, threatened or endangered bird, plant and invertebrate species on Lehua will help to accomplish restoration goals outlined in multiple federal species recovery plans. Restoration also offers opportunities to inform the public about Hawai‘i's native species and efforts to conserve them.

Lehua can serve as a model for demonstrating restoration techniques which will have applications in other areas. Restoring unpopulated islands is one of the most cost-effective and lasting types of habitat restoration. Islands are a manageable size for intensive restoration projects, especially when eradication of an alien species is involved. Eradicating alien species in large areas can be very expensive, logistically challenging, and subject to risks of re-invasion from adjacent areas outside the restoration zone. Lehua, however, is small enough that the rats and the worst of the alien plant species can be completely removed. Furthermore, Lehua's isolation and difficult access help protect it from reinvasion by alien species after restoration has begun. While reinvasion will always be a major concern, it is much easier and cheaper to protect and manage uninhabited islets and islands like Lehua than similar habitats on the larger, populated islands in Hawai‘i.

1.2 Purpose of This Supplemental EA

1.2.1 Description of Selected Alternative in the 2005 EA

Alternative 2, the selected alternative in the FONSI for the original 2005 EA, involved the following actions for meeting the stated goals and objectives:

1) Complete eradication of alien European rabbits using hunting and trapping techniques, followed by

2) removal of Polynesian rats using aerial broadcast of the rodenticide diphacinone (50 ppm active ingredient), with an option to use the rodenticide brodifacoum (25 ppm active ingredient) as a followup the following year, but only if it could be shown that the sole reason for eradication failure was due to the use of the rodenticide diphacinone and no other factor, followed by

3) native plant restoration using a plant restoration and reintroduction plan considering appropriate sources of plants, population genetics, and historic ranges of plants.

4) Throughout the project, efficacy and impact monitoring would occur, as well as implementation of a plan to avoid reintroduction of alien plants and animals.

Both diphacinone and brodifacoum have been approved for conservation use by the U.S. Environmental Protection Agency (EPA). Diphacinone for conservation use in the small, ½" pellet formulation required for Lehua Island has been approved by the Hawai‘i Department of Agriculture. The approved labels for diphacinone and brodifacoum are included as Appendix A. Use of brodifacoum for conservation purposes is considered for this project only if any eradication failure can be attributed directly to the use of diphacinone and not to any other factors. See Chapter 2 for more detailed descriptions of the modified operational plan for eradication of Polynesian rats from Lehua Island and Chapter 3 for more information on diphacinone and brodifacoum and their comparative impacts.

The proposed action for rat eradication as described in the 2005 final EA involved the following actions and mitigation measures. These measures include those required in the July 5, 2005, informal Section 7 consultation with the National Marine Fisheries Service, which resulted in their determination that the project "may affect but is not likely to adversely affect" Hawaiian monk seals (*Monachus schauinslandi*) and sea turtles.

- Rodenticide would be applied by hand or aerial application and/or bait stations, using a hopper [bait applicator] for aerial application with a 120 degree deflector, using hand broadcast in shoreline areas and/or with bait placed directly in burrows or other areas deemed to be high quality rat habitat, establishing a coastal no-fly buffer for bait application, and flying the helicopter from the shoreline inland to minimize risk of bait dropped in the ocean.
- Diphacinone would be applied at 12.5 lb/acre per application and bait stations would be filled with bait continuously for approximately two years, allowing rats free access. Any application of brodifacoum bait would be applied at up to 13.5 lb/acre or less as required.
- Conducting eradication operations during the dry summer season between April and October when rat population densities and the potential for storm events are lowest to avoid bait being washed into the ocean (only when no rain is forecast for 48 hours).

- Time bait broadcast in the summer to avoid shorebird season and juvenile albatross and transient birds of prey.
- Buffer zones within which no bait will be distributed will be maintained around shoreline areas.
- Bait will not be applied in high wind conditions.
- Any crews conducting hand broadcast of rodenticide pellets on the island will maintain a 100-foot buffer from [Hawaiian monk] seals.
- The helicopter will be required to alter course to avoid flying directly over hauled-out seals and no bait will be spread on or around seals.
- Pellets will be evaluated to ensure that no active seeds of nonnative plants are embedded in the bait pellets.
- Monitor plant communities before, during, and after rabbit and rat eradication efforts to determine if alien "weeds" are increasing and implement a weed management program if necessary.

Following completion of the 2005 Final EA for ecological restoration of Lehua Island, European rabbits were eradicated through intensive hunting efforts in 2005 and 2006. With the rabbits gone, the next effort is the eradication of the rats.

1.2.1 Modifications to the Selected Alternative

Since the FONSI was signed in 2005, new information has become available and important modifications to the rat eradication operation on Lehua Island associated with the selected Alternative 2 have been determined to be more effective for rat eradication, while also minimizing and/or avoiding adverse impacts to both birds and humans. Therefore, the USFWS and DLNR, as joint lead agencies, have determined that the original Environmental Assessment for the Lehua Island Ecosystem Restoration Project should be supplemented to evaluate the impacts associated with these modifications (40 CFR 1502.9(c)). The purpose of this supplement is to describe the rat eradication operation for Lehua Island in detail as modified and evaluate the effectiveness and impacts associated with the entire operation, including the modifications.

The changes are:

- Changing the season of starting rat eradication from mid-summer to mid-winter (December through February) in order to:
    - increase efficacy of the operation by exposing 100% of the individual rats to rodenticide because rat breeding is far lower and may cease in winter and the presence of dependent rat pups in burrows insulated from exposure to rodenticides is lowest,
    - substantially decrease exposure of migratory nontarget bird species to rodenticide since fewer birds are present in winter,
    - avoid exposure of fishermen, limpet-pickers, and tourists, who rarely if ever use the area during winter,

  - reduce bird strike hazard concerns for the helicopter pilot by operating when fewer seabirds are present, and
  - avoid all federally listed threatened and endangered seabird species, which are not present on Lehua during the winter.

- The following changes to operational activities and mitigation described in the 2005 EA will be made for two reasons. First, these changes will improve the effectiveness of bait application in critical shoreline areas, thus, providing for 100% exposure of all individual rats to rodenticide bait. Second, they are not necessary to protect marine organisms due to the extremely low risk and toxicity of bait to marine organisms, as shown by the literature summary and analysis in this supplement (Section 3.3.2) and marine sampling results from the February 2008 Mokapu Island rat eradication near Moloka‘i.
  - A deflector on the bait applicator will not be used. Such deflectors, as currently designed, make it difficult for pilots to distribute bait pellets uniformly and frequently cause the bait applicator to malfunction;
  - The helicopter pilot and project manager will be given the discretion to distribute bait in the most effective pattern and will not be required to fly only from the coastline inland toward the ridgeline; and
  - The project manager and the pilot will not be excluded from applying bait adjacent to coastlines, thus ensuring a uniform and complete distribution of pellets in shoreline areas used by rats.
- If any broadcast of rodenticide pellets occurs after black-footed and/or Laysan albatross chicks hatch, then all pellets within 6 feet of the nest will be manually collected so that chicks cannot play with or ingest pellets. All albatross nesting is localized near and at the top of the northwestern portion of the inner crescent, facilitating such removal.
- The definition of “high winds” is clarified to be 35 mph (as stated on the pesticide label), beyond which aerial application of pesticides cannot be conducted.

1.2.3 Scope of this Supplement

This supplement also provides additional details for the rodenticide operation and conducts more detailed impact analyses than was provided in the original 2005 EA. It also clarifies some scientific interpretations regarding the timing of the operation in the original 2005 EA. Updated evaluation of significance of impacts of the rat eradication operation per Hawai‘i HRS 343 is also included. This supplement serves as the final document for the rat eradication operation on Lehua Island and supersedes the 2005 EA in this matter.

This supplement does not:

- Affect the component of selected Alternative 2 regarding the rabbit eradication project, since this project was successfully completed in 2006.
- Modify the program for plant and animal restoration as identified in the original 2005 EA.
- Modify the programs for quarantine of and response to releases of nonnative plant and animal species (Appendix B).

- Duplicate unnecessary information regarding the affected environment and other information, as this information is detailed in Chapter 2 of the 2005 EA.
- Re-evaluate the no action alternative (not conducting a rat eradication project on Lehua Island) or Alternative 3 (use only brodifacoum as the rat eradication rodenticide on Lehua Island) as these alternatives were evaluated and rejected by the USFWS in the FONSI for the 2005 EA dated September 30, 2005.
- Describe the alternatives not considered in detail, as these are described in the 2005 Final Lehua EA.
- Consider or evaluate the use of any other rodenticides, chronic or acute, such as chlorophacinone, zinc phosphide or cholecalciferol for use on Lehua Island.

Therefore, the USFWS, in cooperation with DLNR and USDA-APHIS-Wildlife Services, will use this supplemental EA and other appropriate documents to determine only if the modified rodent eradication might have significant impacts requiring analysis in an Environmental Impact Statement (EIS). No other decisions are necessary for this operation.

The USFWS and the Hawai‘i Department of Land and Natural Resources (DLNR) are joint lead agencies on this EA per NEPA, and DLNR is the approving agency per HRS 343. This supplemental EA is prepared consistent with the National Environmental Policy Act (NEPA), its Council on Environmental Quality (CEQ) implementing regulations at 40 CFR 1500-1508, and HRS 343 and its implementing regulations at HAR 11-200, Department of Interior NEPA manuals 516 DM 1, 2, and 8 (USFWS) and other pertinent Federal and State of Hawai‘i laws and regulations.

The action discussed in this supplement was developed cooperatively by USDA-APHIS-Wildlife Services (WS), USFWS, and DOFAW staff in collaboration with members of the Offshore Island Restoration Committee (OIRC). Operational requirements, monitoring plan, and project planning were also reviewed by the New Zealand Island Eradication Advisory Group as part of the analysis for this supplement, integrating methodologies that have been successful in New Zealand and other locations.

This EA will be in effect through the eradication efforts and into the future if rats ever re-invade Lehua. However, this document would need to be further supplemented if the eradication project is further modified, new information becomes available that indicates that the effects would be different than those anticipated and documented in the original 2005 EA as modified by this supplement, or new eradication technologies become available.

A Finding of No Significant Impact (FONSI) per NEPA is anticipated based on the analysis in Chapter 3 of this supplement. No significant impacts are anticipated per HRS 343.

Details of the general impacts of rats (*Rattus* spp.) on island ecosystems are found in both Chapter 1 of the 2005 final EA for Lehua Island and a more detailed analysis is found in Chapter 1 of the Final Environmental Assessment for Eradication of Polynesian Rats (*Rattus exulans*) from Mokapu Island, Hawai‘i (FONSI signed January 10, 2008). Both final EAs are available from the Point of Contact on the cover of this supplement, and the Mokapu EA is available as a .pdf file at http://www.fws.gov/pacificislands/. This information merely supports and does not change the analyses in this supplement, which supersedes the original 2005 EA regarding the rat eradication project on Lehua Island.

1.3 Public Comments on 2005 EA

For the original 2005 EA, USFWS and DLNR DOFAW contacted all the organizations and individuals identified in Chapter 5 of the original Lehua Island EA. The USFWS and DOFAW made extensive efforts in 2005 to inform and seek input from the general public and government regulatory agencies, regarding the need to restore Lehua Island. In addition, members of a non-profit conservation organization, Island Conservation, were consulted and helped prepare the 2005 EA. A member of the New Zealand Department of Conservation conducted a site visit to Lehua Island and provided input into the development of plans for the eradication of rabbits and rats from the island.

The following comments were obtained regarding the proposed rat eradication operation during the 2005 scoping period:

- Public: two letters in strong support and one not in support (regarding the rabbit eradication project)
- Hawai‘i environmental recreational businesses: two in strong support
- Pacific Seabird Group: strong support.

Based on the input gathered during the 2005 scoping process, a Draft EA was prepared and issued for public comment on June 8, 2005. The Draft EA was posted on the Service's Pacific Islands Office website per agency policy for NEPA and a notice requesting comment was published in the State of Hawai‘i's Office of Environmental Quality Control Bulletin per HRS 343. Letters were also sent notifying interested parties of the availability of the Draft EA and requesting comments. A list of all the parties who were notified is included in Chapter 5 of the Final EA. The 30-day comment period closed on July 8, 2005. Four letters were received: one from The Nature Conservancy (comments in support of the project), and three from State of Hawai‘i agencies: the Historic Preservation Division (concurring with the finding of no adverse impact with mitigation and requesting the final cultural resources report), the Department of Health (no comment), and the Office of Environmental Quality Control (requesting an evaluation of an HRS 343 finding of no significant impact and requesting documentation of contact with Native Hawaiian cultural experts). These letters and the response letters to them are included in Appendix F of the 2005 final EA.

1.4 Results of Section 7 Consultations on the 2005 EA Selected Alternative and on the 2008 Project Modification

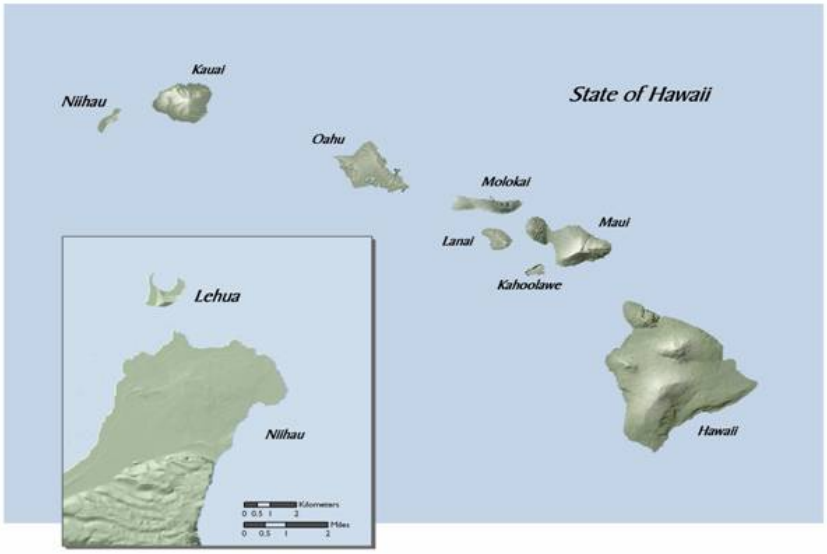
Intra-Service Section 7 Endangered Species Act Consultation for the Newell's shearwater and Hawaiian petrel (both listed), and the band-rumped storm-petrel (a candidate for listing) was finalized in April 2005 and included in Appendix E of the 2005 final EA. The USFWS determined that the proposed action would benefit the ecosystem and the three species of seabirds, resulting in a determination of "may affect but is not likely to adversely affect" the shearwater and petrel, and a determination of "no effect" for the storm-petrel. The following actions were required to reduce adverse effects: *"To minimize disturbance, hunting and trapping of rabbits will occur in the winter, when no listed seabirds are present and the smallest numbers of other seabirds are nesting. Newell's shearwaters, Hawaiian petrels, and band-rumped storm-petrels commute to and from their nesting sites at night. Aerial broadcast by helicopter and hand-placement of rodenticide bait would be done during the day, so no direct disturbance to*

listed seabirds is expected." With the proposed change to a winter operation, when listed seabirds are not present, no impact is anticipated. All bait application operations would be conducted during the day.

An informal Section 7 consultation with the National Marine Fisheries Service (NMFS) (letter dated July 5, 2005, Appendix E of the 2005 EA) resulted in concurrence by NMFS that the proposed eradication projects on Lehua Island were not likely to adversely affect federally listed Hawaiian monk seals or sea turtles. The mitigation measures identified in the letter are included in italicized letters in Section 1.2.1 of this supplement. The letter also concurred with the USFWS statement that *"bait pellets will not present a poisoning hazard to foraging seals or sea turtles."* NMFS further stated: *"It should also be noted that as a result of this project there could be indirect beneficial effects to both monk seals and sea turtles arising from increased native plant cover which will stabilize soils, reduce sediment runoff into the ocean and improve marine water quality. This may result in the establishment of improved nearshore foraging habitat for both monk seals and sea turtles. Given the mitigation put in place under the draft EA we conclude that any effects of the proposed action on monk seals or sea turtles would be discountable. NOAA Fisheries Service therefore concurs with your determination that the project may affect but is not likely to adversely affect ESA listed species under our jurisdiction."*

When asked in April 2008 if the proposed project modifications would require re-opening of the informal consultation, NMFS replied: *"Although the proposed action has been slightly modified (spraying season changed from summer to winter, deflector no longer will be used for spray application), these modifications do not change the effects of the proposed action to ESA-listed marine species. Thus the 2005 concurrence letter is still valid, and there is no need to reinitiate Section 7 consultation*" (L. Smith, NMFS ESA Section 7 Coordinator, Honolulu, email dated April 14, 2008).

Fig. 1. Location of Lehua Island off the coast of Niʻihau and Kauaʻi



Graphic: USFWS

Fig. 2. Lehua Island aerial photograph #1

Photo: Steve Ebbert

Fig. 3. Lehua Island aerial photograph #2

Photo: Google Earth

1.5 Consistency with USFWS and DOFAW Invasive Species Policies

In this supplemental EA, the term "invasive" will be used to mean any nonnative species introduced into an area that causes ecological harm. The key characteristics of an invasive species involve the following factors:

- the human-induced introduction of a species occurring outside of its historically known natural range
- potential dispersal and establishment of the species within the new suitable habitat, and
- resulting damage to the native ecology, the economy, or human health.

Not only are invasive species highly adaptable, but typically they encounter favorable conditions in their new environment, and their rapid establishment can be facilitated by the availability of more or better resources, fewer or less efficient native competitors and predators, and/or a more advantageous habitat (Courchamp et al. 2002).

Restoration of native biological diversity by removing invasive species and preventing further introductions is a major priority of the USFWS, consistent with its mission and USFWS policy for managing refuges for biological diversity, integrity, and environmental health (601 FW 3, 2001).

The USFWS policy as stated in 601 FW 3 (2001) is to, first, maintain existing levels of biological integrity, diversity and environmental health at the landscape scale; and secondly, to restore lost or severely degraded elements of integrity, diversity, and environmental health at the landscape scale and other appropriate landscape scales where it is feasible and supports achievement of refuge purposes and mission. The policy recognizes that applications of chemicals may be necessary to maintain biological integrity. The policy also focuses on preventing the introduction of invasive species, detecting and controlling populations of invasive species, and providing for restoration of native species and habitat conditions in invaded ecosystems.

DOFAW's policy, as described in the Hawai'i's Comprehensive Wildlife Conservation Plan (Mitchell et al. 2005) identifies seven objectives that are necessary for the long-term conservation of Hawai'i's native wildlife of which the first two are related to protection of native species and habitats and management of invasive species:

1) Maintain, protect, manage, and restore native species and habitats in sufficient quantity and quality to allow native species to thrive;
2) Combat invasive species through a three-tiered approach combining prevention and interdiction, early detection and rapid response, and ongoing control or eradication.

Under the first objective, a high priority was identified to remove introduced mammals, including rats, from important habitats to establish ungulate and predator-free areas on each island, including landscape-level predator management.

Under the second objective, high priority actions include continuing coordination of invasive species prevention, management and control programs for county, state, Federal and private sector entities through existing entities and mechanisms, as well as to continue research on effective management methods and tools for introduced vertebrates and other taxa, including rats.

1.6 Previous Hawai‘i Rodent Eradications and Consistency with Executive Orders

Using New Zealand's successes in controlling and eradicating invasive rodents as a model, Hawai‘i has been at the forefront of efforts in the United States to adapt agricultural and commensal rodent control and eradication techniques to native ecosystem conservation areas. Developing rodenticide application techniques and obtaining registrations for them in Hawai‘i has been pursued with the goal of conservation of plants and animals, while allowing natural and active restoration or recovery of species impacted by introduced rodents. This has been carried out by substantially reducing rodent populations in valuable native ecosystems on the main Hawaiian Islands and by eradicating them from uninhabited offshore islands and remote atolls. Beginning in 1990, the USDA-APHIS-Wildlife Services eradicated rats from four remote Pacific atolls where rats were having devastating impacts on seabird colonies (Hess et al. *in press*):

1) Conducted with the USFWS and the Samoan Department of Wildlife and Marine Resources, eradicated Polynesian rats on uninhabited Rose Atoll (17 acres), American Samoa, using brodifacoum (0.005% active ingredient) in bait stations. Although the first attempt controlled but failed to eradicate rats, a subsequent application with bromethalin (0.01% active ingredient), an acute neurotoxin, completed the eradication.
2) Wildlife Services (WS) and the Hawai‘i Department of Land and Natural Resources (DLNR) eradicated Polynesian rats in 1993 from 348-acre Green Island, Kure Atoll (Northwestern Hawaiian Islands; NWHI) using techniques similar to those used on Rose Atoll.
3) WS and U.S. Navy eradicated black rats from Eastern Island (362 acres) and Spit Island (3 acre) at Midway Atoll, using the same techniques used at Rose Atoll for Eastern Island and snap-trapping on Spit Island. They also eradicated rats on 1,300-acre Sand Island at Midway Atoll using bait stations and live traps. Sand Island is the largest and the only inhabited island in the United States from which rats have been removed.

The last attempted eradication on a Pacific Atoll (black rats from Palmyra Atoll, in the equatorial Line Islands in 2001) was by far the most complex, involving approximately 742 acres and 52 islets, most of which were densely vegetated. This operation failed due to insufficient funding, inadequately trained personnel, and interference with bait stations by several species of land crabs.

In 2002, the Offshore Island Restoration Committee (OIRC) was formed to restore selected small offshore islands around the Main Hawaiian Islands. To date, eradication of black rats (*Rattus rattus*) on Mokoli‘i near O'ahu using diphacinone in bait stations has been completed (D. Smith, *pers. comm.*). In February 2008, and the first Hawai`i aerial rodenticide application to eradicate Polynesian rats on an island, using diphacinone, was conducted on Mokapu Island off Moloka‘i.

These past, existing and proposed projects are fully consistent with and contribute to complying with Executive Order 13112 of February 3, 1999, *Invasive Species,* which requires Federal agencies whose actions may affect the status of invasive species to, subject to the availability of appropriated funds and within administrative budgetary limits, use relevant programs and authorities to:

- Prevent the introduction of invasive species;
- Detect and respond rapidly to and control populations of such species in a cost-effective and environmentally sound manner;

- Monitor invasive species populations accurately and reliably;
- Provide for restoration of native species and habitat conditions in ecosystems that have been invaded;
- Conduct research on invasive species and develop technologies to prevent introduction of and provide for environmentally sound control of invasive species; and
- Promote public education on invasive species and the means to address them.

Under Executive Order 13186 of January 11, 2001, *Responsibilities of Federal Agencies to Protect Migratory Birds,* the USFWS is given authority to recognize and promote the great ecological and economic value of migratory birds to the United States and other countries by promoting the conservation of migratory bird populations. The Executive Order states that each Federal agency shall, to the extent permitted by law and subject to the availability of appropriated funds and within Administration budgetary limits, and in harmony with agency missions:

- Support the conservation intent of the migratory bird conventions by integrating bird conservation principles, measures, and practices into agency activities and by avoiding or minimizing, to the extent practicable, adverse impacts on migratory bird resources when conducting agency actions;
- Restore and enhance the habitat of migratory birds, as practicable;
- Prevent or abate the pollution or detrimental alteration of the environment for the benefit of migratory birds, as practicable;
- Design migratory bird habitat and population conservation principles, measures, and practices, into agency plans and planning processes (natural resources, land management, and environmental quality planning);
- Ensure that environmental analyses of Federal actions required by NEPA or other established environmental review processes evaluate the effects of actions and agency plans on migratory birds, with emphasis on species of concern;
- Identify where unintentional take of migratory birds reasonably attributable to agency actions is having, or is likely to have, a measurable negative effect on migratory bird populations, focusing on species of concern, priority habitats and key risk factors.

This supplemental environmental assessment contributes to continuing pursuit of these goals, consistent with Executive Orders 13112 and 13186 and Federal and state policy, by planning and implementing hand and aerial broadcast applications of diphacinone on small offshore islands with established invasive rodent populations to restore the natural habitats of native seabirds and plants.

1.7 Compliance with Laws/Executive Orders Applicable to Rodent Eradication

1.7.1 Coastal Zone Management Act in Hawai‘i

The Coastal Zone Management Act (CZMA) is a Federal law that delegates authority to states with approved management plans, including Hawai‘i, to restore and protect coastal waters and

resources. The Federal regulations at 15 CFR 930 and State statutes, regulations and guidance interact to provide the framework for State management of the coastal resources.

Federal regulations at 15 CFR 930.30-930.46 require "all Federal agency activities, including development projects affecting any coastal use or resource will be undertaken in a manner consistent to the maximum extent practicable with the enforceable policies of approved management plans." "To the maximum extent practicable" is defined as "fully consistent with the enforceable policies of [State] management plans unless full consistency is prohibited by existing law applicable to the Federal agency" (15 CFR 930.32).

"Enforceable Policies" are state policies which are legally binding through state constitutional provisions, laws, regulations, land use plans, ordinances, judicial or administrative decisions, by which a State exerts control over private and public land and water uses and natural resources in a coastal zone and which are incorporated in an approved management plan. They contain standards of sufficient specificity to guide public and private uses, and the state must base any objections to proposed actions within the coastal zone on the enforceable policies (15 CFR 930.11(h)).

The Hawai'i Office of State Planning has the authority to review Federal actions or actions on Federal lands for compliance with the State's implementing law (HRS 205A). The State of Hawai'i law for implementing the federal Coastal Zone Management Act is HRS 205A: Coastal Zone Management.

The following State enforceable policies have been identified as potentially applicable and consistency with these laws is documented in Section 3.4 of this supplement:

- HRS 149A: Hawai'i Pesticides Law
- HRS 195D and HAR 13-124: Conservation of Aquatic Life, Wildlife, and Land Plants (endangered species)
- HRS Chapter 6E: Historic Preservation
- HRS 342D and HAR 11-54: Water Pollution and Water Quality Standards

1.7.2 State of Hawai'i Code for Pesticide Control

In addition to the Federal Insecticides, Fungicides, and Rodenticides Act (FIFRA), under which formulations of both diphacinone and brodifacoum are registered for conservation use, the State of Hawai'i also requires management and registration of pesticides. These requirements (in HRS Chapter 149A, HAR 4-66, 2006), are administered by the Hawai'i Department of Agriculture. The law requires licensing and labeling for pesticides, certification for applicators, and licensing for sales.

Both diphacinone and brodifacoum are considered "restricted use" pesticides. Therefore, pesticide applicators supervising the proposed program must have a Category 2 certification for persons using or supervising the use of pesticides in forests, forest nurseries, and forest seed producing areas. The helicopter pilot doing the bait application must have a Category 4 certification for persons applying pesticides by aircraft.

No person shall apply a restricted use pesticide by aircraft except by special permit under the following conditions and limitations:

- A written application including information on that applicant and applicator, purpose of aerial treatment, pesticide formulation, dosage, method of aerial treatment and proposed number of treatments to be made, and proposed sites and conditions.
- The request for special permit may be refused in writing, with rationale, if it is determined that the proposed aerial treatment may cause unreasonable adverse effects to humans or the environment (meaning any unreasonable risk to humans or the environment, taking into account the economic, social, and environmental costs and benefits of use of the pesticide (4-66-2)) or will create a hazard.
- A special permit specifies the time period and may specify and limit the number of treatments, or continuous treatments when conditions are not expected to change or vary during subsequent treatments conducted in the same designated area or areas.
- The Hawai‘i Department of Agriculture shall be notified 24 hours in advance of the treatment.
- The special permit does not relieve the permittee from the penalty provisions or the law or any liability for any damage or contamination of crops or plants, animals, man and the environment resulting from the aerial treatment.

USDA will obtain the necessary permit for aerial application of the rodenticide on Lehua Island, and all rodenticide applications will be under the direct supervision of a certified applicator.

1.7.3 The Endangered Species Act

The Endangered Species Act (ESA) provides the means to conserve ecosystems upon which threatened and endangered species depend as well as the conservation of endangered and threatened species, and provides for taking steps as may be appropriate for meeting U.S. obligations in treaties and conventions such as migratory bird treaties with Mexico, Japan, Canada and Russia. It prohibits the “take” of listed threatened and endangered animal species without meeting certain procedural requirements. “Take” includes harassment which is defined as an “intentional or negligent act or omission which creates the likelihood of injury to wildlife by annoying it to such an extent as to significantly disrupt normal behavioral patterns which include, but are not limited to, breeding, feeding, or sheltering”(50 CFR 17.3).

Hawai‘i State law HRS 195D-4 and associated regulations at HAR 13-124 govern the State regulation of endangered and threatened species. It provides for all Federally listed species to also be listed by the State, although the State retains the right to uplist species listed as threatened by the Endangered Species Act to endangered status. It also provides a list of endangered species at HAR 13-124.

No adverse impacts to and potential beneficial impacts on listed species were identified during informal Section 7 consultations with the USFWS and NMFS for the 2005 EA. The modification for a winter, rather than a summer, operation eliminated any potential for adverse impacts on listed seabirds and NMFS reaffirmed that the modifications would not change the results of consultation for Hawaiian monk seals and sea turtles. Standard mitigation for avoiding disturbance to monk seals hauled out on land will be followed.

No other marine mammals would be adversely impacted under the Marine Mammal Protection Act.

1.7.4 The Migratory Bird Treaty Act and Executive Order Guidance for Protection of Migratory Birds

The Migratory Bird Treaty Act (MBTA), originally passed in 1918, implements the United States' commitment to four bilateral treaties with Mexico, Japan, Russia and Canada for the protection of migratory bird resources. The Canadian treaty was amended in 1995 to allow traditional subsistence hunting of migratory birds. Each of the treaties protects selected species of birds and provides for closed and open seasons for hunting identified migratory game birds. Although the MBTA applies to the Federal government, based on the D.C. Circuit Court of Appeals decision (*The Humane Society of the United States v. Glickman*, Case No. 99-5309, decided 18 July 2000), other case law has found that the MBTA does not apply to actions, Federal or non-Federal, in which incidental (indirect) take of migratory birds occurs incidental to some other activity conducted for some other purpose. Subsequent to the *Humane Society* decision, the U.S Fish and Wildlife Service issued a Director's Order (now superseded and reinforced by USFWS Manual 724 FW 2, *Migratory Bird Permits*) that clearly applies the MBTA to the Federal government. Federal agencies must obtain permits for the same activities for which permits are required for other entities, including permits for bird banding, scientific collecting permits, and depredation.

The USFWS regulations do not provide for permits for any other type of activity, including the application of pesticides. However, the USFWS decided to prepare an environmental impact statement (EIS) for an initial incidental take permit and a subsequent environmental assessment (EA) for renewal of that permit under MBTA per a California District Court action (civil action number 01-2288) for aerial application of brodifacoum on Anacapa Island, California (National Park Service 2000), even though the Court did not require application of NEPA to such a permit. Therefore, the precedent is set for the application of MBTA permits for aerial application of rodenticides for the purpose of rodent eradication for ecological objectives on land under Federal jurisdiction. However, the USFWS has no formal policy in place regarding the requirement for a permit for pest eradication projects. Therefore, although this document will provide sufficient NEPA analysis for a permit application for adoption (40 CFR 1506.3) by the USFWS should one be needed, the USFWS authority per the MBTA will not require that the Federal government nor anyone else request a permit for any rodent control or eradication projects conducted on Lehua Island.

The USFWS published a list of species not regulated under the MBTA in 2005 (*Federal Register* 70(49): 12710-12716). Although many avian species found in Hawai‘i are native to North America but not to the Hawaiian archipelago, the MBTA does not exempt a species covered by one or more of the four conventions that is nonnative to Hawai‘i but native within the contiguous United States or its territories (same *Federal Register* notice). Of the species found on Lehua, the nutmeg mannikin (*Lonchura punctulata*), the house sparrow (*Passer domesticus*), the rock dove (*Columba livia*), and the zebra dove (*Geopelia striata*) are not protected under the MBTA. The northern cardinal (*Cardinalis cardinalis*), house finch (*Carpodacus punctulata*), barn owl, and cattle egret are nonnative to Hawai‘i but still protected under the MBTA. However, the cardinal and house sparrow are not present on Lehua Island in the winter months. The nonnative barn owl is known to be adversely impacting native birds on Lehua (VanderWerf 2007) and the cattle egret may also be feeding on chicks and eggs and potentially competing for nest sites.

On January 10, 2001, President Clinton issued Executive Order 13186, *Responsibilities of Federal Agencies to Protect Migratory Birds,* requiring that Federal agencies not only support

the conservation intent of the migratory bird conventions, but also identify where unintentional take that is reasonably attributable to agency actions is likely to have measurable negative effects on migratory bird populations.

The analyses for birds protected under the MBTA and requiring analysis under E.O. 13186 potentially present on Lehua Island in the winter are included in this document.

1.7.5 State of Hawai‘i State Wildlife Sanctuaries

Lehua Island is a legally designated State Seabird Sanctuary. Per 13 HAR Chapter 125, the State of Hawai‘i, under the authority of the DLNR, can establish wildlife sanctuaries for the purpose of conserving, managing and protecting indigenous wildlife in sanctuaries. It is prohibited to remove, disturb, injure, kill or possess any form of plant or wildlife or to introduce any form of plant or animal life without a permit. Permits may be issued to enter or land upon identified sanctuaries only for scientific, educational, or conservation purposes and shall specify any terms and conditions deemed necessary for the conservation, management, and protection of indigenous wildlife and wildlife habitats. Therefore, a permit for carrying out conservation operations in a sanctuary will need to be issued by DLNR prior to conducting the rat eradication project on Lehua Island.

The island is also zoned as a Conservation District per HRS 183C and associated regulations at HAR 13-5. Because eradication of alien species is a standard management activity on Conservation lands and no construction or other alterations are proposed, there is no need for a Conservation District Use Permit.

1.7.6 National Historic Preservation Act

Section 106 of the National Historic Preservation Act (NHPA) requires that every Federal agency take into account how each of its undertakings could affect historic properties, and provide the Advisory Council on Historic Preservation (ACHP) a reasonable opportunity to comment on the proposed project. Any property that is listed on or eligible for listing on the National Register of Historic Places, including archaeological resources, is considered historic. The protections of Section 106 extend to properties that possess significance but have not yet been listed or formally determined eligible for listing, as well as properties that have not yet been discovered but possess significance.

The Federal action agency is responsible for initiating and completing the Section 106 review, generally coordinating with the State Historic Preservation Officer (SHPO). The process includes:

- Identifying and evaluating the significance of historic and archaeological properties;
- Assessing the effects based on criteria in 36 CFR 800 (“No Effect”, “No Adverse Effect”, “Adverse Effect”);
- Consulting with the SHPO or ACHP if the agency determines that adverse effects would occur.

HRS Chapter 6E, Historic Preservation, implements the NHPA in Hawai‘i, under the jurisdiction of the DLNR, State Historic Preservation Division. The state law requires that before any agency or officer of the State or its political subdivisions commences any project which may affect historic property, aviation artifacts or a burial site, the agency or officer shall advise the

department and allow the department an opportunity for review of the effect of the proposed project, consistent with Section 6E-43 [prehistoric and historic burial sites], especially those on the Hawai‘i register of historic places. The proposed project shall not be commenced, or in the event that it has already begun, continued until the department shall have given its written concurrence (Section 6E-8). Section 6E-43.6 also regulates the inadvertent discovery of burial sites.

The State Historic Preservation Officer concurred with the USFWS determination that the project will have "No Adverse Effect" on significant historic sites on Lehua Island (letter dated 10/17/05), provided that the following mitigation measure measures are implemented: 1) Submission of a completed archaeological inventory survey report; 2) Recovery of data from a hearth site by a qualified archaeologist; and 3) placement of site tags on historic properties prior to restoration. Mitigation measures 2) and 3) are completed and measure 1) is in progress and will be completed prior to rat eradication.

1.7.7 Magnusen-Stevens Act and Essential Fish Habitat

The Magnusen-Stevens Act provides for protecting certain fish stocks that have declined to the point where their survival is threatened and other stocks that have been so substantially reduced in number that they could become threatened from fisheries and direct and indirect marine, estuarine, and other aquatic habitat losses. Essential Fish Habitat (EFH) identified in Fishery Management Plans required by law includes those waters and substrate necessary to identified stocks of fish for spawning, breeding, feeding, and/or growth to maturity, considering the species full life cycle. An "adverse effect" on EFH means any impact that reduces the quality and/or quantity of EFH, including direct or indirect physical, chemical, or biological alterations of the waters or substrate and loss of, or injury to, benthic organisms, prey species and their habitat, and other ecosystem components. Adverse effects to EFH may result from actions occurring within EFH or outside of EFH, and may include site-specific or habitat-wide impacts, including cumulative impacts. The Federal action agency retains the discretion to make their own determinations as to what actions may fall within NMFS' definition of "adverse effect."

The analysis of potential impacts to EFH is discussed later, with a determination of no adverse affect.

1.7.8 Federal Clean Water Act and State HRS 342D and HAR 11-55

The U.S. Environmental Protection Agency (EPA) has issued a final rulemaking pursuant to the Clean Water Act regarding whether a National Pollution Discharge Elimination System (NPDES) permit is required for application of pesticides that are applied over or near water (71 FR 227:68483-68492, November 27, 2006). The final rule, at 40 CFR 122.3, states that the "application of pesticides consistent with all relevant requirements under FIFRA (*i.e.*, those relevant to protecting water quality), is excluded from the requirements to obtain a National Pollutant Discharge Elimination System permit in the following two circumstances:

"(1) The application of pesticides directly to waters of the United States in order to control pests…

"(2) The application of pesticides to control pests that are present over the waters of the United States, including near such waters, where a portion of the pesticides will unavoidably be deposited to waters of the United States in order to target the pests effectively; for example,

when pesticides are aerially applied to a forest canopy or when pesticides are applied over or near water for control of adult mosquitoes or other pests."

Based on the final rule, this proposed action does not require a NPDES permit because the second of these criteria applies to the proposed bait application at Lehua, which will be in full compliance with FIFRA. The Hawai'i Department of Health's regulations regarding NPDES permits, found in HAR 11-55-04(h), are in full agreement with the language in 40 CFR 122.3.

The State of Hawai'i also has a law and associated regulations for managing and protecting freshwater and marine water quality, located at HRS 342-D and HAR 11-54. Analysis regarding the low potential for water quality degradation under HRS 342-D is included in Section 3.6.2 of this document.

1.7.9 Subsistence and Other Human Uses

ESA and MBTA allow for subsistence take of species protected pursuant to their authority. Analysis of potential impacts to subsistence users in the Hawaiian Islands is incorporated into Chapter 3.

Executive Order 12898 *Federal Actions to Address Environmental Justice in Minority and Low Income Populations* (1994) requires every Federal agency to collect, maintain, and analyze information assessing and comparing environmental and human health risks borne by populations identified by race, national origin or income. To the extent practical and appropriate, the Federal agency shall use this information to determine whether its actions and programs have disproportionately high and adverse human health or environmental effects on minority populations and low-income populations.

No studies were found regarding ongoing cultural practices on Lehua Island. No comments regarding cultural uses were received in response to the request for comments on the 2005 Draft EA for the Lehua Island Ecosystem Restoration Project. However, responses gathered during interviews by DOFAW for the 2005 Lehua Island EA indicated that residents from both Kaua`i and Ni`ihau visit the waters around Lehua to fish. Interviewees said that the residents of Ni'ihau visit the island whenever the water is good; residents of Kaua'i apparently visit Lehua less frequently, most likely due to the distance from Kaua'i. Respondents reported that people visit the island in order to fish and to collect opihi (marine limpets) and limu (seaweed).

The waters around Lehua are also a destination for SCUBA trips departing from Kaua'i. Lehua's remoteness makes this trip a full-day undertaking, so use is light compared to most dive sites in Hawai'i. Sportfishing, bird watching, snorkeling, and eco-tourism also occur in the waters around Lehua. All these activities tend to occur in the calm summer season when the waters between Kaua'i and Lehua are not as rough.

As almost all human use on and around Lehua occurs in the summer and the proposed modification changes the operational season to winter, when the surrounding seawaters are rough, no adverse impacts are expected to human use. Based on field and laboratory tests and experiences with past broadcasts, toxicants are not expected to accumulate in fish or marine invertebrates. Therefore, no closures of Lehua for fishing and gathering for consumptive purposes are planned if diphacinone is used. The public will be notified prior to diphacinone application and the results of laboratory tests for diphacinone residues in Lehua seawater and marine species will be made public as soon as they become available. However, a temporary closure would be considered if brodifacoum is used, in addition to public notification, which

could go into the summer fishing season. Therefore, no impact associated with diphacinone use would occur regarding either subsistence use of resources or disproportionate impacts to minorities or low income communities and no further analysis is conducted in this supplement. However, the possible closure mitigation for brodifacoum is included is discussed later, even though the chance of using this rodenticide is low and would only be considered if diphacinone fails to eradicate rats from Lehua.

1.7.10 Consistency with the Hawai‘i State Comprehensive Wildlife Conservation Plan

The Hawai‘i Comprehensive Wildlife Conservation Plan (Mitchell et al. 2005) was prepared by the Hawai‘i Department of Land and Natural Resources (DLNR) as a requirement for participating in the State Wildlife Grant program administered by the USFWS. It presents strategies for long-term conservation of Hawai‘i’s native terrestrial and aquatic species and their habitats. The Plan built upon Hawai‘i’s strong history of conservation and involved working with resource managers, biologists, and concerned individuals statewide.

The mission of Hawai‘i’s Comprehensive Conservation Strategy is to guide conservation efforts across the state to ensure protection of Hawai‘i's wide range of native wildlife and the diverse habitats that support them.

The Plan identifies and analyzes threats to Hawai‘i’s Species of Greatest Conservation Need (SGCN), including all native terrestrial animals, all endemic aquatic animals, additional indigenous aquatic animals identified as in need of conservation attention, a range of native plants identified as in need of conservation attention, and all identified endemic algae. All the species evaluated in this supplement except the cattle egret, glaucous-winged gull (*Larus glaucescens*), peregrine falcon (*Falco peregrinus*), northern cardinal, house finch, nutmeg mannikin, and house sparrow are identified as SGCN in this Plan.

Consistency of the proposed action with the Plan is integrated into this supplemental EA wherever it is appropriate. Therefore, this rat eradication project on Lehua Island as modified is fully consistent with and contributes to implementing the Hawai‘i Comprehensive Wildlife Conservation Plan.

1.7.11 Consistency with the County of Kaua‘i General Plan Objectives and Policies

The County of Kaua‘i General Plan goals for environmental quality seek to achieve an ecological balance between a high quality of life and an environment in which the natural resources of the island are viable and sustainable, maintain and, if feasible, improve the existing environmental quality of the island and to control pollution. The stated policies applicable to the proposed action, with associated policies, include:

Chapter 3. Caring for Land, Water, and Culture.

> Policy 3.1.1.1(d). Projects undertaken with State or County lands or funds shall be designed to conserve heritage resources.
>
> Policy 3.3.2.1. Preserve important archaeological and historic sites.

The project as modified is fully consistent with and contributes to implementing the applicable objective and associated policies.

1.7.12 Native Hawaiian Rights

Native Hawaiians have special rights under Federal law, the State Constitution, and State statutes, as interpreted by Federal and State courts. Under the State Constitution, the State and Counties are empowered to promote the health, safety, and welfare of all inhabitants without discrimination as to ethnic origin. The State and Counties recognize the rights of native Hawaiians and the laws concerning land and waters that have been established through the State Constitution, State and Federal Laws, and State and Federal court decisions:

- Native Hawaiian water rights provided under State Water Code, HRS Chapter 174C.
- Kuleana lands, water rights, and access rights provided under the Kuleana Act of 1850, as recognized in current statutes, rules and court decisions.
- Konohiki and hoa'aina fishing rights provided under the 1839 Law of Kamehameha, as modified by subsequent legislative acts and court decisions.
- Traditional and customary rights of native Hawaiians, such as for access and gathering, provided under the State Constitution and Hawai‘i revised statutes, as interpreted by the courts (for example, the *PASH* case).
- Burial rights provided under the Hawai‘i Historic Preservation Act and the Federal Native American Graves Repatriation Act.
- Preservation of historic properties and archaeological resources provided under the Federal Archaeological Resources Protection Act of 1979, the National Historic Preservation Act of 1966, and the Hawai‘i Historic Preservation Act.

The proposed project will have no impact on any native Hawaiian rights to land, access, burial rights, or rights to resources. The impact of the program to freshwater and marine fish, invertebrates, and associated consumption of marine fish are evaluated in Section 3.3.

2.0 DESCRIPTION OF THE MODIFIED PROJECT AND MITIGATION

2.1 Selection of Winter Timing for Application of Rodenticides

Since the operational objective is to eradicate Polynesian rats from Lehua Island, a key consideration when evaluating potential timing is the biology of the target rat population. It is especially important to identify periods when rat reproduction is low or nonexistent so that dependent juveniles are not in burrows where they will not be exposed to the rodenticide (Orueta and Ramos 2001). Consideration of the abundance of rats and their seasonal food availability is also important.

Subsequent to the consideration of rat biology, the presence of nontarget species that could be vulnerable to rodenticide exposure and toxicity, either directly by eating bait or indirectly by eating prey that have rodenticide residues within their tissues must be evaluated. Selecting the season when most nontarget species are not present is the most effective mitigation method (Orueta and Ramos 2001). In Hawai‘i, and especially on arid Lehua where the weather varies little, with storms occurring occasionally in the winter, weather is a tertiary consideration.

The proposed timing in the 2005 EA was based on the common sense but erroneous assumption that rat reproduction would peak during the wet winter months when water, sprouting plants, insects, and other food items would be most available. However, rodent population monitoring on Lehua in 2007 and 2008 demonstrated that rat populations and breeding activity are actually highest in dry summer months and lowest in winter (Dunlevy 2008).

Lehua rat abundance and reproductive status were monitored in July and September 2007 and March 2008 in preparation for the eradication operation (Dunlevy 2008). Standardized traplines were put in place to sample microhabitat types from coast to summit in order to make inferences regarding Polynesian rat distribution. In July and September, captures occurred from the coast to the summit in all habitat types, and large numbers of rats, which are typically nocturnal, were seen active during the day. In March, only one capture of an adult pregnant female occurred, on the coast, and only two rats were seen active during the day. The corrected trap index, a comparative index of rat abundance based on the number of rats trapped per the number of trap nights, was 30% in July, 17% in September, and 1% in March. The best predictor of trap success was the presence of nearby vegetation. Rats are distributed throughout the island, reinforcing that the entire land area must be treated, with special attention paid to vegetated areas.

Dunlevy (2008) concluded that rat numbers on Lehua dropped significantly from the summer through the fall and apparently reached a low sometime during the winter months. In the summer months, almost 50% of the population was composed of juvenile rats (indicating a high level of breeding at that time), dropping to about 30% in the fall. No juveniles were caught in March, although the only rat trapped was a pregnant female, indicating that breeding was occurring at that time. As population and reproduction levels on Lehua are apparently lowest during the winter, the winter provides the highest probability for successful eradication of the rats. Tamarin and Malecha (1972) postulated that the most probable environmental factor controlling breeding is the length of daylight.

Based on the site-specific findings on Lehua Island (Dunlevy 2008), the probability of eradication success is greatly increased by conducting the operation in December through

February (with follow-up if needed in March), when reproduction and the probability of juvenile rats in burrows is clearly low or non-existent.

The timing the operation in winter avoids disturbing the largest numbers of birds (especially the wedge-tailed shearwaters), all the listed bird species, and the thus the majority of the vulnerable eggs and chicks (see Table 1 below). This also resolves many concerns with the exposure of nontarget species to the rodenticide. Applying the rodenticide when most nontarget species are absent is the primary and most assured method of reducing the exposure of these species to the toxicant or disturbance (Orueta and Ramos 2001). Low numbers of birds flocking in the air also reduces safety concerns associated with helicopters striking birds. Based on surveys conducted on Lehua from 2002 through 2005, the greatest abundance of native bird species is present from March through August and many of the overwintering birds are non-nesting visitors (VanderWerf et al. 2007).

Table 1. Bird species present/absent on Lehua during winter (December-February) and winter breeding status (B = winter breeder, NB = winter non-breeder)

Species	*Present*	*Absent*
Black-footed albatross (B in low numbers)	X	
Laysan albatross (B in low numbers)	X	
Hawaiian black noddy (NB)	X	
Great frigatebirds (NB)	X	
Brown booby (B in low numbers)	X	
Red-footed booby (NB)	X	
Red-tailed tropicbird (B in low numbers)	X	
Sooty tern (NB, rare visitor)	X	
White-tailed tropicbird		X
Gray-backed tern		X
Wedge-tailed shearwater (most numerous Lehua species; 25,000 pairs breeding in summer)		X
Newell's shearwater (threatened species)		X
Christmas shearwater		X
Bulwer's petrel		X
Hawaiian petrel (endangered species)		X
Band-rumped storm petrel		X
Pacific golden plover (NB, migrant)	X	
Ruddy turnstones (NB, migrant)	X	
Glaucous winged gull (NB rare visitor)	X	

Peregrine falcon (NB rare visitor)	X	
Barn owl (NB, alien)	X	
Cattle egret (NB, alien)	X	
Great blue heron (rare visitor)		X
Black-crowned night heron (rare visitor)		X
Rock dove (alien)		X
Zebra dove (alien)		X
Nutmeg mannikin (alien)		X
House sparrow (alien)		X

However, certain species are primarily present only in the winter (migratory Pacific golden-plover and ruddy turnstone and the two species of breeding albatross) and will warrant extra caution when planning and conducting operations. Older albatross chicks in particular may peck at or swallow objects near their nests. However, albatross chicks in January and February do not yet move from the nests so the proposed mitigation (removing the pellets near nests with chicks, all of which are localized near the top of the western portion of the inner crescent), would reduce any concerns.

In general, storms occur most frequently from October through March, with occasional heavy rains and sometimes strong winds. Average wind speeds are highest during the summer trade-wind period. From September through April, when trade winds are not as prevalent, wind speeds in excess of 12 mph occur about 40% of the time. Frequent light variable winds are balanced by occasional very strong winds. Most storms occur during the winter but are usually short-duration events (http://www5.ncdc.naoo.gov/climatenormals/clim60/states/clim_HI_01.pdf).

For the Lehua operation, the primary weather-related logistical constraints are wind and rain. Rodenticide application will not be conducted in winds higher than 35 mph. For each application day, a forecast of five days and nights without significant rainfall (>13 mm) is preferred (Dunlevy 2007). Currently, the closest long-term weather station with similar conditions is located on the leeward side of Kaua‘i in Kekaha, with weather data collected from 1949 through 2000. The average precipitation during the spring, summer, and fall (April through November) varies from 0.31 inches to 2.78 inches. The average precipitation for December is 4.13 inches, for January is 4.05 inches, for February is 2.22 inches and for March is 2.06 inches (Western Regional Climate Center). The National Weather Service in Honolulu will be used to supply forecasts for the Lehua area, and a rain gauge and anemometer will be set up on site and recorded daily before and after bait application (Dunlevy 2007).

Therefore, the ideal time to conduct the rodent eradication project on Lehua Island would be at the time of year that ensures the highest probability of successfully distributing rodenticide and eradicating rats while having the lowest potential impact on nontarget species. Between December and March, most species of native seabirds that may provide food for rats and are also nontarget species are absent from Lehua or only present in low numbers. Only the red-footed and brown-footed boobies are present in any numbers, and only albatrosses have chicks,

although small, and all the nests are located within 60 to 300 feet below the summit on the western portion of the inner crescent.

Therefore, the optimum timing of the operation is based primarily on the lack of rat reproduction and the absence of the majority of seabirds. This occurs during the winter months from December through February. Operations may continue into March, if necessary.

2.1.1 Rodenticide Selection and Use

Selection of the most appropriate rodenticide for the specific conditions of a project is one of the primary decisions for any rodent eradication project. Rodenticides must be used in the lowest quantity and toxicity which ensures that every rodent is exposed to a lethal dose while minimizing adverse environmental effects, especially impacts to nontarget species. Prudent use is also critical to ensure that regulators will allow effective rodenticides to continue to be made available for future use (Marsh 1985, Cromarty et al. 2002).

Marsh (1985) advised selecting the rodenticide for which the target rodent has a high susceptibility and nontarget wildlife species have a low susceptibility, thereby maximizing effectiveness and minimizing adverse effects, especially to nontarget species. Maximizing effectiveness of the selected rodenticide involves combining the critical factors of the concentration of the active ingredient in the bait formulation, the method of application, the bait application rate, and the seasonal timing of bait application (when rodent populations, reproduction, and alternative foods are lowest) to ensure that all target rodents are exposed to a lethal dose. Both the selection of the appropriate rodenticide and the technical considerations must also consider the complexity of the physical terrain and the size of the island to be treated.

The technical considerations of efficacy are more straightforward than those involved in minimizing adverse effects on nontarget species and other public trust environmental resources. Minimizing overall adverse effects is possible in a variety of ways; most mitigation methods for reducing hazards to nontarget species involve (Kalmbach 1943, Marsh 1985):

- Applying bait when nontarget species are not present, present in seasonally low numbers, or not breeding or raising young;
- reducing bait toxicity to nontarget species;
- reducing the acceptance of bait (exposure) by nontarget species;
- minimizing or avoiding exposure of nontarget species (e.g., via protective stations);
- minimizing rodenticide residues in the tissues of target and nontarget species.

In summary, the selection of the appropriate rodenticide in an effective bait formulation for a specific project must ensure a high potential for efficacy in eliminating invasive rodents when conducted according to the description of the proposed action during the optimum seasonal time frame, while having the lowest potential for adverse impacts to nontarget species.

The New Zealand Department of Conservation (NZ DoC) implemented a policy in October 2000 that placed restrictions on the use of brodifacoum for conservation purposes on the New Zealand mainland because of documented levels of direct and indirect poisoning of nontarget species. NZ DoC conducted a study using diphacinone 0.005% formulations of pellets and blocks in mainland control situations that demonstrated the efficacy of diphacinone in the field (Gillies et al. 2006). Studies in Hawai‘i have also documented the efficacy and lower nontarget impacts of

diphacinone in field and laboratory studies (Swift 1998, Dunlevy et al. 2000, Dunlevy and Campbell 2002, Nelson et al. 2002, Spurr et al. 2003a and 2003b, Eisemann and Swift 2006).

For the rodent eradication project on Lehua, the rodenticide with the lower risk to nontarget species, diphacinone, has been selected for use. Brodifacoum would be used only if the application of diphacinone fails and the failure can be determined to have been caused by the rodenticide diphacinone itself and not improper or inadequate application methods, timing, bait life, bait competition with nontarget species, or other operational issues.

2.1.2 Operational and Ecological Monitoring

Introduction

Monitoring the efficacy of rodent eradication and successful ecosystem restoration, as well as environmental fate and the potential for adverse effects on nontarget species and populations is critical to rodent eradication projects (Atkinson 1994, Courchamp et al. 2002, Smit 2003). Smit (2003) focuses on the importance of monitoring not only to determine if goals are achieved, but also to add to existing knowledge on how to better manage ecosystems, including learning from experience and adjusting actions when necessary to better meet objectives. He states that it is critical to define indicators that characterize the state of the resource, define the intensity of monitoring, and use thresholds to determine whether to increase or decrease the intensity of monitoring or stop it altogether, based on the results of monitoring. Courchamp et al. (2002) also emphasize the importance of learning from "unwitting mistakes made in the past, since all results contribute to an understanding of island ecology and can be used in future conservation actions on other islands."

Bait Monitoring

Rodenticide uptake by target rodents must be evaluated to ensure that sufficient bait is applied to ensure consumption of a lethal dose by 100% of the rats (Sterner and Ramey 2002). Monitoring of bait take during broadcast application requires refined monitoring techniques (Sterner and Ramey 2002). Careful testing and calibration of equipment and methods prior to broadcast and detailed records of the amounts of rodenticide applied and the areas (using Differential GPS systems) over which it is distributed are the first steps in the monitoring of bait application, while providing for the computation of nominal bait application rate. Monitoring the appropriate density of bait is also necessary. In addition, broadcast applications should monitor bait degradation, which should also be outlined in detail within the specific project operation plan. In general, this entails closely monitoring weather conditions in representative habitats and areas of possibly variable exposure and observing how rapidly the bait deteriorates. The level of toxicant in the bait should also be monitored, both before application and once on the ground, to ensure that all rats are exposed to the appropriate dosage of active ingredient for meeting eradication objectives (Spurr and Powlesland 2000).

On-the-ground application monitoring methods are outlined in detail in the specific project operation plan (Dunlevy 2007). It is planned that rodenticide application will be assessed by measuring and recording the total amount of bait applied and evaluating the actual bait distributed on the ground in the treatment area using ground surveys. The number of pellets found within census plots will be recorded immediately after bait application, while recording

substrate and slope. To assess bait disappearance, marked pellets will be examined daily for up to 14 days until they disappear or biodegrade.

Eradication Efficacy Monitoring

Radio telemetry will be used to monitor the fate of 20 rats fitted with radio collars before the operation begins. Signals will be monitored for three days before bait application to affirm activity and until all collared animals are confirmed dead post treatment. Recovered rats will be necropsied to determine exposure to rodenticide and cause of death, and carcasses will be individually labeled, bagged and frozen for residue analysis.

Rat presence post-operation will be assessed using rodent traps, using the protocols established during the 2007 and 2008 Lehua rat surveys. An appropriate number of transects with snap-traps will be laid out and baited daily for several days after pre-baiting to avoid rats' natural fear of new objects. Monitoring for success in meeting the eradication objective will be conducted in July 2009 and 2010 using night-vision goggles, chew blocks, snap-traps, and tracking tunnels, as appropriate. Rat presence will be assessed annually in the summer for two years post-operation (Dunlevy 2007).

A brodifacoum formulation could be used only if operational failure occurs and can be determined to have been caused by the rodenticide diphacinone itself and not improper or inadequate application methods, timing, bait life, bait competition with nontarget species, or other operational issues. If this were to occur, the brodifacoum product would be used the following winter, at least one year after the diphacinone treatment, during the same time period. The treatment regime would be similar, entailing two broadcasts following the approved label. The primary difference between the application of diphacinone and brodifacoum would be the application rate dictated by the label.

Ecological Monitoring

Monitoring for primary and secondary adverse impacts on nontarget species is one of the foremost concerns for rodent eradication projects. Sometimes the primary factor in determining whether to conduct an eradication project is the evaluation of the ecological cost of killing individuals of nontarget species, and potentially adversely impacting populations, as compared to the benefits associated with meeting ecosystem restoration objectives. Primary hazards (through direct ingestion of bait) and secondary hazard (through eating prey with rodenticide residues in their tissues) to individuals of nontarget birds may potentially occur. The evaluation and determination of killing a proportion of a nontarget population and whether it would cause adverse impacts at a population level must be considered in terms of species' biology and population dynamics. Based on the analyses in these sections, no adverse impacts to any bird species are anticipated.

Baseline vegetation and bird surveys have been conducted on Lehua Island (Wood et al. 2004, VanderWerf et al. 2007) and will be continued following the eradication operation to monitor restoration success. Key indicators of successful restoration will be improvements in the status of threatened plant species and native vegetation abundance and composition, as well as recolonization by nesting seabirds. The spread of introduced plants from reduced herbivory by rabbits and rats will also be monitored. A comprehensive list of introduced plants on Lehua, documenting qualitative and quantitative weed information (Wood et al. 2004) provides the comparative baseline.

Populations of desired nontarget species, including nesting seabirds and protected plants, will be actively monitored for a sufficient period to produce reliable estimates before and after operations. At a minimum during the operation, personnel will collect all carcasses found incidentally for necropsy and laboratory analysis of rodenticide residues in tissues. Any rat carcasses found in the open will also be recorded and collected for residue analysis. Avian predators or scavengers seen on Lehua will also be recorded. The cattle egret, which is known to be an opportunistic predator on eggs and chicks, and the barn owl, recently recorded on Lehua, are both introduced species to Hawai‘i.

Multiple seawater and intertidal invertebrate and fish tissue samples will be collected after the broadcast and sent to at least two laboratories to test for the presence of rodenticide residues. The exact timing of sample collection will be determined by safety considerations, but the goal will be to collect two sets of post-application samples, 24 hours and 7 days after each bait application.

2.1.3 Rodenticide Label Requirements for Invasive Rats

All applications of rodenticides must follow label requirements as approved by the US Environmental Protection Agency (EPA) pursuant to FIFRA.

EPA-Approved Diphacinone Label

The FIFRA Section 3 label (see label in Appendix B) for conservation purposes (EPA reg. no. 56228-35, Diphacinone--50, 0.005% or 50 ppm active ingredient), has the following use requirements:

- Broadcast applications are prohibited on vessels or in areas of human habitation. Broadcast bait pellets by helicopter or manually at a rate of 11.1 to 13.8 kg/ha (10 to 12.5 lbs/ac) of bait per treatment. Depending upon local weather conditions, make a second broadcast application (typically 5 to 7 days after the first application), at a rate no higher than 13.8 kg/ha (12.5 lbs/ac). In situations where weather or logistics only allow one bait application, a single application may be made at a rate no higher than 22.5 kg/ha (20 lbs/ac). Aerial (helicopter) applications may not be made in winds higher than 35 mph. If rodent activity persists after application, set up and maintain tamper-resistant bait stations or apply bait directly to rodent burrows in areas where rodents remain active. If terrain does not permit the use of bait stations or burrow treatment, continue with broadcast baiting, limiting such treatment to areas where active signs of rodents are seen. Maintain treatments for as long as rodent activity is evident in the area and rodents appear to be accepting bait.
- For all methods of baiting, monitor the baited area periodically and collect and dispose of any dead animals found.

Broadcast applications of Diphacinone--50 at the maximum label rate of 22.5 kg/ha (20 lb/ac) result in approximately one 2 to 3-gram pellet distributed about every square meter.

EPA-Approved Brodifacoum Label

The nationwide label (see Label in Appendix B) approved by EPA for conservation purposes (EPA reg. no. 56228-37, Brodifacoum-25D, 0.0025% or 25 ppm active ingredient) has the following use requirements:

- Broadcast applications are prohibited on vessels or in areas of human habitation. Broadcast bait using aircraft, ground-based mechanical equipment, or by gloved hand at a rate no greater than 18 kg bait/ha (16 lbs/acre) per application. Make a second broadcast application, typically 5 to 7 days after the first application, depending on local weather conditions, at a rate no higher than 8 lbs. of bait per acre (9 kg bait/ha). In situations where weather or logistics only allow one bait application, a single application may be made at a rate no higher than 16 lbs. bait per acre (18 kg/ha). Aerial (helicopter) applications may not be made in winds higher than 30 knots (35 mph). Pilot in command has final authority for determining safe flying conditions. However, aerial applications will be terminated when the following conditions are present: Windspeed in excess of 25 knots with an evaluation of the terrain and impact of the wind conditions and not to exceed a steady wind velocity of 30 knots. Set the application rate according to the extent of the infestation and apparent population density. For eradication operations, treat entire land masses.
- Assess baited areas for signs of residual rodent activity (typically 7 to 10 days post-treatment). If rodent activity persists, set up and maintain tamper-resistant bait stations or apply bait directly to rodent burrows in areas where rodents remain active. If terrain does not permit use of bait stations or burrow baiting, continue with broadcast baiting, limiting such treatments to areas where active signs of rodents are seen. Maintain treatments for as long as rodent activity is evident in the area and rodents appear to be accepting bait.
- Monitor the baited area periodically and, using gloves, collect and dispose of any dead animals and spilled bait properly.

The maximum broadcast application rate of Brodifacoum-25D allowed by the label is 18 kg/ha (16 lb/ac), resulting in a density of approximately one two-gram pellet per square meter.

2.1.4 Necessary Permits for Eradication Projects on Lehua Island

For conducting any actions on Lehua, which is designated as a State Wildlife Sanctuary, DOFAW must issue a permit (HAR 13-125-6).

For aerial application of rodenticide on Lehua, a permit from the Hawai‘i Department of Agriculture per HRS 149A and HAR 4-66 must be acquired prior to beginning the operation.

If diphacinone fails to achieve eradication and the decision is made to use brodifacoum, it could only be applied if the State Department of Agriculture’s Pesticides Branch also licenses the FIFRA Section 3 label for use within Hawai‘i under HRS Chapter 149A.

2.2 Aerial Application of Rodenticides

2.2.1 Overall Application Operational Plan

Rats will be removed using a rodenticide formulation containing the active ingredient diphacinone at 50 ppm. The bait is dyed green by the manufacturer to reduce acceptance by birds. The rodenticide will be uniformly broadcast across the emergent land area of the island at an approved application rate exposing all rats to a lethal dosage. Rodenticide bait will be applied

once all necessary personnel and equipment are in place and a suitable weather forecast is received.

Application on Lehua will be completed by aerial broadcast across 100% of the land area of the island. All rodenticide application would be carried out under the direct supervision of licensed pesticide applicators. Aerial broadcast will be carried out utilizing an agricultural spreader suspended from a helicopter. Bait will be applied at a nominal rate of 10 lb/ac (11.25 kg/ha) in at least two separate broadcast applications to be carried out approximately five to seven days apart. To ensure as uniform an application rate as possible, onboard Differential Geographic Positioning System (DGPS) in the helicopter and computerized GIS mapping would document the application area. This allows real time and after-the-fact monitoring and assessment of the rodenticide application, as well as printouts showing the actual path covered by the helicopter during bait application. Immediately prior to the application, all equipment will be tested and calibrated in a location allowing for repairs or adjustments and ensuring accurate application results.

The first application is planned to occur after December 1, 2008 and before the end of February 2009. If broadcast is delayed beyond this period, it will be attempted again the following winter. Each aerial broadcast application operation will start as early in the day as possible to provide as much time as possible to finish the entire application, check GPS printouts and re-apply to any gaps and conclude bait application monitoring before dark.

Weather forecasts will again be consulted before deciding on the appropriate day for the second application of bait, five to seven days after the first application, using the same application rate and methods outlined above. The five-to-seven day interim before the second application may be extended if sufficient bait is still on the ground (greater than 5 lb/ac bait remaining). Flight lines for the second application may be treated in reverse and/or perpendicular to the first application. Up to four such applications, if necessary, will comprise the full treatment regimen. Treatment should be completed by March if possible, or by the end of March at the latest.

If rats persist post-operation and it is shown that the active ingredient diphacinone is solely responsible for the failure (as opposed to application methodology, weather or bait condition, for example), bait containing 25 ppm brodifacoum could be used the following winter per the approved label. With the exception of label differences, the treatment would be the same as that described in this section for diphacinone per the brodifacoum label. However, this is not expected to be needed.

2.2.2 Bait Handling, Storage and Staff Safety Measures

- All possible measures to transport and store the rodenticide in a manner that maintains its integrity and quality will be followed. Optimum storage conditions are a cool, dry and dark environment.
- The rodenticide will be inspected regularly, and the relative humidity within the storage shed monitored. Any bait with evidence of decay will be immediately removed and disposed of according to the label, and the remaining bait dried. Anti-moisture techniques will be used for stored bait as needed, including use of moisture absorbents, ventilation during dry conditions, elevating and maintaining drainage around storage facility.

- Staff will follow all approved label handling and disposal instructions, such as:
    - Storing bait in original containers tightly sealed in a dry secure place inaccessible to unauthorized people, children and pets, away from fertilizer and products with strong odors, which may contaminate the bait and reduce acceptability.
    - Wearing long-sleeved shirts, long pants, gloves and shoes plus socks at all times when handling bait;
    - Wearing required personal protective equipment (PPE) such as eyewear and dust masks when loading bait for aerial application;
    - Washing hands and all exposed skin before eating and after work;
    - Not reusing empty bait containers for any reason, and disposing of empty bait containers according to the label;
- Any spilled bait on land will be collected for disposal according to the label.
- In the event of a helicopter ditching or other event that causes a bait spill into the ocean in a shallow coastal area, bait pellets will be removed from the water and disposed of if it is feasible and safe to do so. Each bucket load should be no more than 500 pounds of bait.

2.2.3 Reporting, Project Debriefing and Adaptive Management

Upon completion of each broadcast, a debriefing will be conducted with all operational personnel, including the pilot, for the purpose of evaluating the application and making any necessary modifications. Upon completion of the project, at a minimum, an internal report will be completed. In addition, a project debriefing will be conducted and lessons learned from this project will be applied to subsequent rodent eradication projects using aerial broadcast in Hawai‘i.

2.3 Resource-Specific Mitigation Measures

Many mitigation measures for project-level actions are already incorporated directly into the description of the eradication operation, including the use of a rodenticide with reduced toxicity to nontarget organisms (diphacinone), conducting the operation in the winter when most nontarget bird species are not present and rodent biology is favorable, safe bait handling procedures, not flying in high winds or when heavy rains are predicted, public notification prior to application, and pre- and post-project monitoring. The following mitigation actions are in addition to those already incorporated into the modified eradication operation and are based on analyses documented in Chapter 3 and included in the Section 7 consultation with NMFS. These mitigation measures will be implemented as part of the operation and are included in the operational plan.

2.3.1 Species on Lehua Protected under the Endangered Species Act

Per the results of the informal Section 7 consultation conducted with the USFWS for the rat eradication project on Lehua Island, the only listed or candidate species that could be present during a summer application would be the threatened Newell’s shearwater, the endangered Hawaiian petrel, and the candidate band-rumped storm-petrel. None of these birds or any other listed birds would be present in the winter (VanderWerf et al. 2007). Per the results of the

informal consultation conducted with NMFS in 2005 and revisited in 2008 based on the modifications to the eradication operation, the following mitigation measures for the endangered Hawaiian monk seal are required:

- The helicopter will be required to alter course to avoid flying directly over hauled out seals and no bait will be spread onto seals.
- Any personnel operating on the ground for monitoring before, during, and after a bait application will always attempt to maintain a 100 foot buffer from seals.

Both NMFS and the USFWS recognized that the eradication operation will benefit listed species by improving vegetation cover and eliminating depredation by rats.

2.3.2 Archaeological Sites Protected under the National Historic Preservation Act

The State Historic Preservation Officer has concurred with the USFWS determination of "No Adverse Effect" on significant historic sites on Lehua Island from the project (letter dated 10/17/05), based on completion of the following mitigation measure: 1) Submission of an approved and completed archaeological inventory survey report; 2) Recovery of data from a hearth site by a qualified archaeologist; and 3) placement of site tags on historic properties prior to restoration. All these measures will be completed prior to rat eradication.

2.3.3 Coastal Zone Management Act and Enforceable and Administrative Policies

The Hawai‘i State Office of Planning has determined that all proposed rodenticide projects must go through the consistency process. The analyses are included in this supplement and are incorporated into the CZM review package. The CZMA review and the public involvement process will be conducted concurrently with the review for this supplement.

2.3.4 Protection for Albatross Chicks from Ingesting Bait

If bait is applied after chicks have hatched, all bait pellets within 6 feet of nests with chicks will be manually removed as soon as possible after bait application. Pellets further than 6 feet away cannot be reached by the chicks sitting in the nest, since they would not yet be mobile.

2.3.5 Human Health

Public notices will be posted and published in local newspapers informing people before the bait is applied. Weather permitting, seawater, marine invertebrate, and fish tissue samples will be collected 24 hours and 7 days after bait is applied to test for rodenticide residues and test results will be published in Kaua‘i newspapers. Use of inland areas of Lehua is by DOFAW permit only. The area is used by recreational divers and limpet and algae gatherers during the summer. However, as the project will be conducted in the winter (December through February, with the potential for some follow-up into March), no potential for impacts would occur. Access permits for other than authorized personnel will not be issued during pre-operational monitoring, distribution of bait, post-operational monitoring and, for diphacinone, one month after the last bait application. If the use of brodifacoum becomes necessary, a temporary harvest closure after bait application could occur if required by the State Department of Health.

2.3.6 Water Quality

In the event of a helicopter ditching or other event that causes a bait spill into the ocean in a shallow coastal area, the State Department of Health would be notified and bait pellets would be removed from the water and disposed of if it is feasible and safe to do so. Each bucket load would hold no more than 500 pounds of bait. See Section 3.2.1 for analysis of the impacts of the loss of a maximum of 500 pounds of bait into the water.

3.0 ENVIRONMENTAL CONSEQUENCES

3.1 Introduction

This chapter includes the technical background and affected environment information for each issue considered in detail, and documents the impact analysis for each issue. This chapter also includes consistency analyses with the Hawai‘i Enforceable and Administrative Policies under the Coastal Zone Management (CZMA), analysis of impacts to birds protected by the Migratory Bird Treaty Act and required by E.O. 13186, and potential impacts to Essential Fish Habitat under the Magnusen-Stevens Act and state equivalent laws. Since the analyses required for the impacts under the identified laws are functionally equivalent to those required for NEPA, these analyses are incorporated into this chapter and are identified as such to facilitate understanding the impacts and resultant determinations and to avoid unnecessary paperwork, consistent with NEPA (40 CFR 1501.7, 1502.25, 1506.4).

To assist understand of the analyses of impacts caused by rodenticides on each issue, Appendix A of this document summarizes the scientific literature regarding the rodenticides diphacinone and brodifacoum and compares their characteristics and their relative toxicity to invertebrates, fish, birds and mammals. It also summarizes the methodologies used in this EA for evaluating the impacts of proposed actions on the resources of Lehua. This information was not included in the 2005 EA and is intended here to help the reader better understand the logic of the impact analyses and how the differing characteristics of the rodenticides apply to those impacts. For additional background, the approved pesticide labels for diphacinone and brodifacoum are included in Appendix B.

Table 2 has also been added below as a reference. It outlines the acute oral doses and dietary toxicity for birds and primary and secondary hazards for birds and mammals as well as known tissue residues for brodifacoum and diphacinone (from Erickson and Urban 2004). In order to understand Table 2 and subsequent risk analyses, it is necessary to understand the following three terms:

- Acute oral toxicity or LD_{50}– A single dose that is lethal to 50% of the test subjects in the population or study group under consideration, expressed as milligram(s) of active ingredient per kilogram of test subject body weight;
- Dietary toxicity or LC_{50}– The concentration of rodenticide in the diet (multiple feedings) that is lethal to 50% of test subjects in the population or study group under consideration, expressed as parts per million of the daily diet.
- Lowest observed effects level or LOEL– The lowest dosage at which measurable effects, such as increased blood-clotting times, are documented. This is not a mortality threshold and no negative impacts are necessarily derived at this hazard level. Diphacinone has LOELs calculated for birds and mammals; brodifacoum does not because of its substantially higher toxicity.

Table 2. Nontarget Hazards to Birds and Mammals from Brodifacoum and Diphacinone Pellets (both at 50 mg of active ingredient (a.i.) / kg bait)[1]

		Brodifacoum	Diphacinone
Acute Oral Toxicity (LD_{50}) to Birds	Mallard	0.26 mg ai/kg	3,158 mg ai/kg
	Northern bobwhite	Not reported	>400, <2,000 mg ai/kg
Acute Dietary Toxicity (LC_{50}) to Birds	Mallard	2.0 ppm	906 ppm
	Northern bobwhite	0.8 ppm	>5,000 ppm
Bird: Primary Hazard from Direct Ingestion	LD_{50} (amount of ai per kg body weight to kill 50% of population)	0.26 mg ai/kg	>400 mg ai/kg
	25-g bird: grams of bait LD_{50} / % of daily food intake	0.13 g / 2.1%	200 g / >100%
	100-g bird: grams of bait LD_{50} / % of daily food intake	0.52 g / 5.4%	800 g / >100%
	1000-g bird: grams of bait LD_{50} / % of daily food intake	5.2 g / 9.6%	8000 g / >100%
Bird: Secondary Hazard from Indirect Ingestion	Blood retention time (half life)	7.3 days	17.5days
	Liver retention time (half life)	217 days	90 days
Bird: Nontarget Incidents	# nontarget incidents reported	252 incidents	6 incidents
Mammal: Primary Hazard from Direct Ingestion	LD_{50} (amount of ai per kg body weight to kill 50% of population)	0.4 mg ai/kg	2.3 mg ai/kg
	25-g rodent: grams of bait LD_{50} / % of daily food intake	0.2 g / 5.2%	1.2 g / 32%
	100-g rodent: grams of bait LD_{50} / % of daily food intake	0.8 g / 9.6%	4.6 g / 55.4%
	1000-g mammal: grams of bait LD_{50} / % of daily food intake	8.0 g / 11.6%	46.0 g / 67%
Mammal: Secondary Hazard from Indirect Ingestion	Blood retention time (half life)	7.3 days	0.82 days
	Liver retention time (half life)	217 days	90 days
Avg. Number of LD_{50} Doses Consumed by Rats by Time of Death	Choice test	40	Not reported
	No choice test	80	
Anticoagulant Residue Levels in Primary Consumers exposed to 50 mg ai/kg bait	Range of whole-carcass residues	2.07-25.97 ppm	0.48-3.4 ppm
Mammal: Nontarget Incidents	# nontarget incidents reported	157 incidents	29 incidents

[1] All data and information from Erickson and Urban (2004)

3.2 Potential Impacts to Soil, Water, Invertebrates and Fish

3.2.1 Environmental Fate of Brodifacoum and Diphacinone in Soil and Water

Both diphacinone and brodifacoum have extremely low solubility in water and bind tightly to organic matter in soil, where the rodenticide is degraded by soil micro-organisms and exposure to oxygen and sunlight. The half-life in soil is 30 to 60 days for diphacinone, and 84 to 175 days for brodifacoum, depending on the soil type. Microbial degradation is dependent on climatic factors such as temperature and the presence of microbes enabling degradation. Therefore, degradation time will increase in colder climates and decrease in warmer places like Hawai`i

(Eason and Wickstrom 2001, Eisemann and Swift 2006). Due the non-polarity of brodifacoum and diphacinone molecules and the ionic strength of seawater, water solubility of both these compounds is extremely low. The solubility of brodifacoum is likely in the low parts per billion range (Primus et al. 2005), with diphacinone assumed to be substantially less soluble.

The low risk of rodenticide showing up in seawater was also demonstrated by sampling conducted after the aerial application of diphacinone bait pellets to Mokapu Island in February 2008. Samples of surface seawater (as well as intertidal limpets and nearshore fish) were collected to address public concerns about contaminating marine life and to verify assumptions that the project would have no negative impacts to marine waters and organisms (see complete Mokapu sampling and laboratory report in Appendix C). These assumptions were based on data from extensive laboratory and field trials submitted to Hawai‘i Department of Agriculture's Pesticides Branch and EPA during the rodenticide registration process. In addition, operational safeguards built into the aerial broadcast process minimized risk of bait pellets getting into the adjacent seawater. These safeguards included applying bait only during sufficiently low wind speeds or when no significant rainfall was predicted, and using a calibrated bait delivery system to avoid overapplication of bait and an on-board differential GPS system to correctly target bait application.

Mokapu Island samples were sent for testing to the U.S. Department of Agriculture National Wildlife Research Center in Fort Collins, Colorado and to the U.S. Geological Survey Columbia Environmental Research Center in Columbia, Missouri.

Results from the laboratories were obtained in April and May 2008. No diphacinone residues were detected in any of the seawater, limpet or fish samples (see Appendix C for results and description of laboratory quality assurance/quality control procedures). This indicated that project mitigation measures, low water solubility of diphacinone, rough winter seas, dilution, or some combination of these factors resulted in little or no rodenticide being released into the water column.

The threat of an accidental spill of rodenticide pellets is a remote possibility. In the event of serious flight difficulties requiring an emergency landing, the helicopter pilot would likely need to jettison the spreader bucket before landing, potentially resulting in up to 500 pounds of bait pellets going into the water. Should such an unlikely scenario occur, the project emergency plan would be enacted, notifying all relevant persons and initiating the appropriate response and clean-up, if possible. However, since the pellets contain only .005% of active ingredient of diphacinone (or .0025% active ingredient in the case of brodifacoum), the actual amount of active chemical ingredient entering the water from a 500 pound bait pellet spill would be less than half an ounce for either rodenticide. Due to the very low water solubility of both rodenticides, very little of this small amount of active ingredient would dissolve into the water column and the risk to marine organisms would be minimal.

Water quality data collected after a massive brodifacoum spill into nearshore waters supports this statement. In 2001, a truck went off the road into the ocean on the east coast of New Zealand's South Island, prior to an eradication project. Twenty tons of 0.002% (20 ppm) brodifacoum bait was spilled into the ocean at a single point. Furthermore, because the seas were calm, the congealed bait material remained on the ocean floor for about a week, until it was diluted and dissipated by wave action. Despite expectations that significant concentrations of brodifacoum

would be dissolved into the water column, brodifacoum levels in water samples were no longer detectable 36 hours after the spill had occurred (Primus et al. 2005).

In summary, the potential for contamination of surface water, groundwater or seawater is extremely low for both diphacinone and brodifacoum. Lehua does not have any known permanent surface water or groundwater. Possible mechanisms for rodenticide to reach the ocean include pellets bouncing off or rolling down steep slopes, being blown off course by high winds, or being washed into the ocean by heavy rains before they are eaten by rats. The last two possibilities will be minimized by not applying bait pellets in high winds (greater than 35 mph) or when heavy rains are forecast. Contamination of ocean water is unlikely due to the same combinations of factors that resulted in the inability of labs to detect rodenticide residues in water samples taken after the Mokapu Island aerial application and the New Zealand bait spill.

3.2.2 Effects of Diphacinone and Brodifacoum on Marine Invertebrate and Fish Species, including Essential Fish Habitat

Marine organisms can be exposed to rodenticides in one of three ways: they can eat bait pellets, they can eat prey items that have accumulated rodenticide in their tissues, or they can absorb rodenticides that have dissolved in seawater through their skin.

Previous sections discussed project mitigation measures to keep bait out of the water, which will minimize risks of marine invertebrates and fish being exposed to rodenticides through any of these pathways. The very low water solubility of both diphacinone and brodifacoum, discussed above, further decreases the likelihood of exposure of marine organisms to dissolved rodenticides.

This section presents evidence that direct ingestion of bait and consumption of contaminated prey are also very unlikely. Evidence includes results from Lehua field observations indicating that nearshore fish are unlikely to be attracted to bait pellets, in addition to sampling results from a rat eradication recently conducted at Mokapu Island, which found no detectable rodenticide residues in marine tissues after two diphacinone applications. Further evidence comes from the unexpectedly low rodenticide levels in marine organisms following a massive 20-ton spill of brodifacoum pellets into shallow, nearshore waters in New Zealand.

The 20-ton spill of brodifacoum in New Zealand documented by Primus et al. (2005) is a "worst case" scenario that will be used here for a highly conservative analysis of rodenticide impacts. These data are conservative because:

- Brodifacoum is more toxic, persistent and bioacummulative than diphacinone; and
- The likelihood of that volume of any rodenticide being spilled into the environment at a point source is extremely remote. The only circumstance under which such a spill could happen in the Hawaiian Islands would be if a vessel carrying large quantities of bait to an island to be treated would sink in shallow nearshore waters, which is highly unlikely, even in the winter.

This analysis will conclude that the risks to marine species at Lehua are very low, based on the lack of likely exposure pathways; the fact that the Mokapu Island bait application did not result in detectable rodenticide residues in marine samples; and the surprisingly low levels of localized

contamination resulting from the worst-case scenario of the New Zealand brodifacoum spill. No significant impacts are anticipated to Lehua's marine invertebrates and fish from the use of either diphacinone or brodifacoum.

Additionally, no physical changes would occur to any Essential Fish Habitat (EFH) at Lehua and the proposed project is not anticipated to adversely affect Essential Fish Habitat in any way. As a result, no EFH assessment per the Magnusen-Stevens Act is required.

Marine Invertebrates

Since diphacinone and brodifacoum are highly insoluble in water, invertebrates could not be exposed to significant amounts of dissolved rodenticides. Therefore, as with the fish, any problems or concerns with invertebrates would have to be caused by their eating bait pellets or eating contaminated prey.

Because many marine invertebrates scavenge or graze on items on the bottom or in intertidal areas, it is possible that they would pick up bait pellets or pellet fragments prior to the pellets breaking down in the water. Complete breakdown of a pellet in the water would likely take only a few days, especially if the water is rough. Therefore, dietary exposure to pellets would have to occur during the few days when the pellet was still intact. The question then becomes whether or not this potential exposure pathway is significant.

Evidence against the existence of a significant dietary exposure pathway for invertebrates, at least in the context of the proposed Lehua project, comes from field sampling of marine invertebrates conducted following an actual rodenticide application in Hawai`i, and another round of sampling done after an accidental New Zealand spill of large amounts of brodifacoum into the ocean.

The sampling program conducted at Mokapu Island, following aerial application of diphacinone bait, did not detect diphacinone residues in any of the water or tissue samples collected. Seawater, limpet and fish samples were collected at Mokapu Island on February 17, 2008, 11 days after the first rodenticide application and 5 days after the second and final application. Two Moloka'i fishermen and a USFWS employee collected samples by hand (water and limpets) and with hook-and-line (fish) after accessing the island by boat. Forty intertidal limpets (*Cellana exarata*) were collected from three locations around Mokapu. Limpets were shelled and the whole bodies, including gut contents, were analyzed for diphacinone residues. Six fish (3 different species) were also collected. Appendix C contains the laboratory reports documenting that no diphacinone was found in the limpets or in the fish muscle tissues. Since gut contents were included in the limpet samples, it can be assumed that because they did not have any bait pellet fragments in their digestive tracts they either did not encounter or did not like bait pellets.

In 2001, a semi-trailer truck went off the road into the ocean on the east coast of the South Island of New Zealand prior to an eradication project. Twenty tons of 0.002% (20 ppm) brodifacoum bait was spilled into the nearshore environment at a single point (Primus et al. 2005). Samples of marine invertebrates and fish were taken immediately after the spill, then monthly for four months, then at three and six month intervals for the following 21 months. Bait spilled into the water began to soften and disintegrate quickly, but the plume of green water from the bait dye lasted approximately 24 hours. Approximately one week post-spill, the congealed grain bait material on the ocean floor was diluted and dissipated by wave action. Most exposure of marine

invertebrates occurred within approximately 300 feet of the spill site; minor exposure was detected from 300 to 900 feet from the spill site, and none was detected beyond 900 feet.

The following results were found during sampling (Primus et al. 2005):

- Mean brodifacoum concentrations in mussels peaked at 0.41 ppm one day after the spill and were just above detection limits after 29 days. Five mussel samples collected 353 days after the spill still averaged 0.002 ppm.
- Abalone gut and muscle tissue residues were highest on day 29 with 0.07 ppm for gut tissue and 0.03 ppm for muscle tissue. At day 191, residues averaged 0.003 ppm for gut and 0.0015 ppm for muscle. At day 353, abalone gut and muscle tissues were 0.0017 ppm and 0.0014 ppm, respectively.
- Limpet tissue maintained detectable brodifacoum residues for about 80 days.

The New Zealand spill was a worst-case scenario but still only resulted in low levels (less than 1.0 ppm) of tissue contamination, mostly within 300 feet of the spill site. However, the persistence of brodifacoum in the tissues was thought to be due to a combination of the high volume of brodifacoum introduced into the shallow marine environmental at one location, a prolonged half-life of the brodifacoum in the invertebrates, and re-exposure to the high volume of bait due to tidal action.

Because brodifacoum would only be considered for use on Lehua if diphacinone fails and the likelihood of a major bait spill into the ocean is minute, the risk of any such persistent accumulation of brodifacoum in invertebrate tissues at Lehua is small.

The effects of rodenticides on corals have not been tested. However, corals would not likely be exposed to rodenticide since coral cover around Lehua is very sparse, due largely to strong wave action. Also, the pellets and even most pellet fragments are too big for the filter-feeding coral polyps to eat and the solubility of rodenticides in water is very low.

For all these reasons, no adverse impacts to marine invertebrates are predicted as a result of using diphacinone or brodifacoum bait pellets on Lehua.

Marine Fish

Since diphacinone and brodifacoum are highly insoluble in water, fish could not be exposed to significant amounts of dissolved rodenticides. Therefore, as with the invertebrates, any problems or concerns with fish would have to be caused by their eating bait pellets or contaminated prey.

In order to address the question of whether fish would eat bait pellets, the USFWS conducted field trials on Lehua Island in 2004, using placebo bait pellets similar in size, shape and material to pellets that might actually be used (C. Swenson, USFWS, unpublished data). Results showed that although certain species routinely inspected bait pellets in the water, none of the 21 nearshore fish species observed ate the placebo bait (Table 3). Although other fish species are present at Lehua that were not observed during these tests, results included a representative sample of species and provided good evidence that fish don't consider bait pellets to be palatable. In any event, bait pellets are not available to fish or other organisms for long since they quickly soften and break up in water, particularly when the ocean is rough (Empson and Miskelley 1999).

If fish aren't exposed to dissolved rodenticides and don't eat bait pellets, the only remaining question is whether they could take up rodenticide by eating contaminated prey items. Strong supporting evidence that prey species would not likely be contaminated comes from field sampling of fish and invertebrates conducted following an actual rodenticide application in Hawai`i, and another round of sampling done after an accidental New Zealand spill of large amounts of brodifacoum into the water.

The sampling program conducted at Mokapu Island, following aerial application of diphacinone bait, did not detect diphacinone residues in any of the water or tissue samples collected. Seawater, limpet and fish samples were collected at Mokapu Island on February 17, 2008, 11 days after the first rodenticide application and 5 days after the second and final application. Two Moloka'i fishermen and a USFWS employee collected samples by hand (water and limpets) and with hook-and-line (fish) after accessing the island by boat. The fish collected included four blue-lined snappers (*Lutjanus kasmira*), one hogfish (*Bodianus bilunulatus*), and one bridled triggerfish (*Sufflamen fraenatus*). All of these fish are shoreline-associated predators that feed primarily on invertebrates and/or small fish. Appendix C contains the laboratory reports documenting that no diphacinone was found in fish muscle or limpet tissues.

Additional supporting evidence for the lack of significant pathways for rodenticide accumulation in fish tissues comes from results of sampling conducted following a massive, 20-ton spill of brodifacoum pellets into shallow, protected coastal waters in New Zealand. Expectations were that significant contamination of fish would result. However, the only fish with detectable residues was a butterfish sampled 9 days after the spill. This fish had only 0.040 parts per million (ppm) brodifacoum in the liver and 0.02 ppm in the gut, and no detectable residues in muscle tissues. No brodifacoum residues were detected in four other fish samples collected between day 14 and 16 after the spill (Primus et al. 2005). As discussed above, brodifacoum was found in invertebrate tissues in concentrations below 1.0 ppm, primarily within 300 feet of the spill site. The New Zealand example was a worst-case scenario but still only resulted in low levels of localized tissue contamination.

For all these reasons, no adverse impacts to marine fish are predicted as a result of using diphacinone or brodifacoum bait pellets on Lehua.

Table 3. Attraction of nearshore marine fishes to placebo Ramik Green rat bait pellets (2-3 gram size) at Lehua Island, Hawai`i, September 18-19, 2004 (USFWS unpublished data)

Common English Name	Scientific Name	Total Number of Fish	Number of bait interactions observed (some individuals interacted multiple times)			Number of bait interactions per species
			Inspected Bait	Touched Bait	Consumed bait	
Orangespine Unicornfish	*Naso literatus*	13	10	8	0	18
Convict Tang	*Acanthurus triostegus*	8	0	0	0	0
Whitebar Surgeonfish	*Acanthurus leucopareius*	85	19	0	0	19

Orangeband Surgeonfish	*Acanthurus olivaceous*	7	3	5	0	8
Achilles Tang	*Acanthurus achilles*	2	0	0	0	0
Ringtail Surgeonfish	*Acanthurus blochii*	1	0	0	0	0
Eyestripe Surgeonfish	*Acanthurus dussumieri*	1	0	0	0	0
Lagoon Triggerfish	*Rhinecanthus aculeatus*	1	1	0	0	1
Black Durgon	*Melichthys niger*	6	21	13	0	34
Pinktail Durgon	*Melichthys vidua*	5	13	9	0	22
Moorish Idol	*Zanclus cornutus*	1	0	0	0	0
Ornate Butterflyfish	*Chaetodon ornatissimus*	1	0	0	0	0
Longnose Butterflyfish	*Forcipiger longirostris*	1	0	0	0	0
Cornetfish	*Fistularia commersonnii*	1	0	0	0	0
Gray Reef Shark (juv.)	*Carcharhinus amblyrynchos*	1	1	0	0	1
Blackspot Sergeant	*Abudefduf sordidus*	1	3	0	0	3
Manybar Goatfish	*Parupeneus multifasciatus*	2	0	0	0	0
Blue Goatfish	*Parupeneus cyclostomus*	3	0	0	0	0
Yellowstripe Goatfish	*Mulloidichthys flavolineatus*	1	0	0	0	0
Hawaiian Hogfish	*Bodianus bilunulatus*	1	1	1	0	2
Parrotfish spp.	Family *Scaridae*	2	0	0	0	0

3.3 Potential Impacts to Humans

Human harvest near Lehua focuses on marine fish and limpets. The analysis in Section 3.2 shows that there is minimal risk that the project will contaminate marine organisms. Field data

collected from Lehua supports the assumption that Hawai‘i nearshore fish do not eat the type of bait pellets planned for use and, therefore, would not have rodenticide residues in their tissues (Table 3). Exposure levels of marine invertebrates to rodenticide, if any, would be at such low levels and for such a short time that no tissue accumulation is anticipated and, therefore, no effects to human consumers are anticipated. As discussed earlier, no diphacinone residues were detected in the seawater, limpets, or fish sampled following the 2008 Mokapu Island rat eradication (see Appendix C). Following the large New Zealand bait spill, only low levels of brodifacoum were detected in organisms close to the spill site.

In addition, access to the waters surrounding Lehua is often risky or impossible for recreational or harvesting purposes during the rough winter months when the bait application would occur. Therefore, collection of limpets and fish is highly unlikely during the period of operations. Project mitigation methods to prevent or minimize bait pellets falling into the water include not applying bait in high winds and not applying before heavy rains that could wash pellets into the water. For all these reasons, the risks of either direct or indirect human exposure to rodenticides in marine organisms are minimal to non-existent. Nonetheless, the public will be notified prior to any bait application. Sampling of water, fish and invertebrate tissues is planned after application, if ocean conditions permit safe sample collection. Results from marine sample testing will be published in Kaua‘i newspapers as soon as they become available.

Harvest or consumption of terrestrial resources, such as plants or seabirds living on the island is illegal without a permit from DOFAW and is not known to occur.

Project personnel would follow all required safety and product handling procedures and would not, therefore, be exposed to harmful amounts of rodenticides.

3.4 Potential Impacts to Birds

Most birds found on Lehua are seabirds, which are present in significant numbers only in the summer and fall and are absent or greatly reduced in numbers in the winter. However, some species are year-round residents. Nonnative passerine birds are also found on Lehua but have only been observed in the summer. Nonnative barn owls are apparently a recent year-round resident. All species on Lehua except the nonnative house sparrow and the nutmeg mannikin are protected under the Migratory Bird Treaty Act.

In general, birds can only be exposed to rodenticides in two ways: either they can eat the bait pellets (direct ingestion) or they can eat prey organisms that have been contaminated by eating rodenticide (indirect ingestion). The types of birds at highest risk of rodenticide poisoning are birds of prey or scavengers that may feed on live or dead rodents that have already eaten rodenticide pellets. However, because almost all the birds on Lehua during the winter operation are seabirds, there is little risk of either direct or indirect rodenticide ingestion by birds. Seabirds do not generally eat things they find on land, such as bait pellets or rodents. Seabirds only eat fish and other marine organisms they catch in the ocean, often far from shore (see Table 4).

Nonetheless, the following sections present data on the effects on birds of direct and indirect bait ingestion. The common theme is that diphacinone, regardless of how ingested, is less toxic than brodifacoum. In most cases, it would be physically impossible for birds to eat enough diphacinone pellets or tainted prey to cause death. As stated earlier, diphacinone is the preferred compound for use on Lehua. Brodifacoum would only be used as a last resort if a failure to

eradicate Lehua's rats could be directly traced to a problem with using diphacinone. Even though it is more toxic than diphacinone, it is unlikely to cause problems since birds are not likely to eat bait pellets or contaminated prey.

3.4.1 Impacts to Native Seabirds Present on Lehua in the Winter

Biology and Status

The numbers of seabirds on Lehua are reduced in the winter compared to the rest of the year, largely because the most numerous species, the wedge-tailed shearwaters, are absent in winter. Breeding is also greatly reduced in the winter and the number of active nests at this time is relatively small. Species observed nesting during the December-February project period (also see Table 1) include both albatross species, brown boobies, and red-tailed tropicbirds. Other year-round Lehua residents like black noddies and red-footed boobies may be breeding in small numbers also but have not been observed to do so. All Lehua seabirds feed on marine organisms offshore and do not gather any food on land.

The following seabird bird species have been recorded on or near Lehua during the winter (VanderWerf et al 2007):

- black-footed albatross
- Laysan albatross
- red-tailed tropicbird (possible year-round resident)
- brown booby
- red-footed booby (year-round resident)
- great frigatebird
- glaucous-winged gull (rare visitor)
- sooty tern (rare visitor)
- brown noddy (rare visitor)
- Hawaiian black noddy (year-round resident)

Potential Impacts from to Seabirds Direct Ingestion of Rodenticide (Primary Nontarget Hazard)

Because the adults of all the Lehua seabird species feed by foraging for fish and other marine organisms offshore (Table 4), it is highly unlikely that any of the seabirds would be attracted to or incidentally pick up bait pellets of either diphacinone or brodifacoum during a winter operation. Few pellets would actually fall into the nearshore waters and any pellets falling into the water would disintegrate rapidly. However, as older albatross chicks in the nest are known to be curious and pick up small articles near the nest, it is possible that a chick could ingest a pellet.

If an adult seabird picked up bait pellets, which is highly unlikely, a black noddy, the smallest of the seabirds, would have to consume 860 grams (2 pounds) of 50 ppm diphacinone bait (based upon the lower reported acute oral LD_{50} of >400 mg/kg body weight for bobwhites) to obtain an LD_{50}-equivalent dosage. It would be physically impossible for such a small bird consume that much bait in one or even several days. An adult red-footed booby, the most numerous seabird

species on Lehua in the winter, would have to consume 8,000 grams (approximately 17.6 pounds) of diphacinone bait, which is physically impossible.

The great frigatebird would have to eat 10,800 g (almost 24 pounds) of 50 ppm diphacinone bait to consume a lethal dose. However, the projected LOEL (extrapolated from the lowest reported LOEL for diphacinone in birds, 0.11 mg/kg/day, Saverie et al 1977) of diphacinone for the great frigatebird, is 0.15 mg/kg/day or about three grams of bait per day (Table 5). As long as bait is present in a treated area, a non-lethal level of exposure like this would be physically possible, although it is highly improbable that any of the seabirds would forage on bait pellets along the coastline rather than fish in the open ocean.

Based on the acute oral LD_{50} figure reported for mallards (0.26 mg/kg body weight), a 108 g (3.8 oz.) black noddy, the smallest species of seabird likely to be present during the operational window, would only have to consume 1.1 gram of 25 ppm brodifacoum bait, or half of one 2-g pellet, to obtain an LD_{50}–equivalent dosage. The average adult great frigatebird weighs approximately 1,350 g (3 lbs) and would need to ingest 14 g, or about seven small-size (2 g) pellets of a brodifacoum product to obtain the LD_{50}–equivalent dosage of 0.35 mg (Table 5). LOEL values are not available for brodifacoum because of its high toxicity. Again, it is highly improbable that any of the seabirds would forage on bait pellets along the coastline rather than fish in the open ocean.

However, it is possible that albatross chicks, known to be curious about objects near their nest, might pick up and inadvertently ingest bait pellets that it can reach from the nest. Albatross chicks grow rapidly after hatching, but assuming a chick in February weighs 660 g (1.5 pounds) (Auman et al. 1997), a chick would have to ingest 5,280 g (over 11 pounds) of diphacinone bait, which is physically impossible. A chick would have to eat 0.07 mg active ingredient diphacinone/kg/day or 1.5 g of bait per day.

This same size chick would need to ingest 6.9 g, or about three or four (2 g) pellets of a brodifacoum product to obtain the LD_{50}–equivalent dosage of 0.17 mg (Table 5). As stated in Section 3.2.1, LOEL values are not available for brodifacoum because of its high toxicity.

Larger albatross chicks would have to ingest proportionately larger volumes of either bait to cause an effect. However, because of the potential for direct ingestion, all pellets within 6 feet of any active albatross nest will be manually removed soon after bait application.

In conclusion, the potential for any adverse impacts to seabirds from consuming either diphacinone or brodifacoum pellets is low because seabirds feed on marine organisms, not bait pellets, and they feed in the open ocean far from where bait will be applied. The possible exception to this is albatross chicks accidentally feeding on bait pellets near their nest. Therefore, mitigation measures include quickly removing bait pellets near any active albatross nests.

Potential Impacts to Seabirds from Indirect Ingestion of Rodenticide (Secondary Nontarget Hazard)

Another potential route of exposure to rodenticides for seabirds is consumption of prey items that have themselves ingested rodenticide. However, all species of seabirds on Lehua consume fish or squid offshore. As a result, it is highly improbable that adult seabirds would feed on or bring fish with rodenticide residues back to their chicks, because the fish in the open ocean would not be exposed to rodenticides and, even if they were, are not expected to feed on bait pellets and

thus bioaccumulate residues, as discussed earlier. Therefore, this scenario will not be evaluated in detail. Nonetheless, the number of grams of marine animal tissues necessary for secondary poisoning to seabirds is included in Table 5. Using the numbers in this table, even under the extreme circumstances of an accident involving a large-scale brodifacoum bait spill and assuming that the seabirds eat nearshore invertebrates (an unknown behavior for the seabird species on Lehua) rather than fish and squid in the open ocean, the risk of mortality for any species of seabird on Lehua is essentially zero for either a diphacinone or brodifacoum formulation.

Table 4. Biological Characteristics of Seabirds Present on Lehua Island in the Winter

Species[1]	Mass (g)[1]	Energy Dynamics	Winter Distribution	Diet	Biological Information	Seasonal Presence in Lehua Area	Citations[2]
Black-footed albatross ka‘upu	Adult: 2800 Chick: <660 g (May)	Data not available	Outside of Japan, 95% breed on Laysan Island and Midway Atoll; breeding recently confirmed on Lehua (VanderWerf 2007), pelagic rest of year	In Hawai‘i, squid, deep-water crustaceans, fish and flyingfish eggs	Age at first breeding >5 years; 1 egg; nest in scooped out hollows; both parents incubate, brood, feed chick	Eggs laid in November and chicks fledge in June and July	Mitchell et al. 2005; VanderWerf et al. 2007
Laysan albatross mōlī	Adult: 2400 Chick: <660 g	Data not available	Breed throughout NWHI and on Kaua‘i, O‘ahu, and Lehua Islands in winter, pelagic rest of year	In Hawai‘i, squid, deep-water crustaceans, fish and flyingfish eggs	Age at first breeding 8 or 9 years; 1 egg; nest scrape to ring-like structure comprised of sand, vegetation, and debris on steep rocky areas on Lehua; both parents incubate, brood, feed chick	Eggs laid between November and December; chicks fledge in July; 1 egg	Mitchell et al. 2005; VanderWerf et al. 2007
Brown booby ‘ā	1340	141 g/day	Little known about movements outside of breeding season	Forages on fish by diving into the water	Age at first breeding 4 to 5 years; 2 eggs/season; nests on ground; both parents incubate, brood, and feed chicks	Breeding from March through May, with fledging by September	Mitchell et al. 2005; VanderWerf et al. 2007
Red-footed booby ‘ā	1000	Data not available	Breed throughout NWHI, Kaua‘i, Kaneohe Bay O‘ahu, Moku Manu and Lehua	In Hawai‘i, flyingfish and squid, mackerel scads, saury, and anchovies	Age at first breeding 3 -4 years; nest in shrubs and trees; 1 egg; both parents incubate, brood and feed chick	Egg-laying possibly February, most young fledged by September’ some birds present year-round	Mitchell et al. 2005; VanderWerf et al. 2007
Great frigatebird ‘iwa	1350	147 g/day	Outside breeding season, breeding adults remain relatively close to breeding area; young and nonbreeders disperse	Steals food from other seabirds and forages for fish by dipping into the water	First breeding at 8 to 10 years; 1 egg/season; platform nests in low bushes; both parents incubate, brood, and feed; females often only breed every 2 to 4 years	Does not breed in the main Hawaiian Islands but can be present and possibly roosting throughout the year; nesting not confirmed on Lehua	Mitchell et al. 2005; VanderWerf et al. 2007

Species[1]	Mass (g)[1]	Energy Dyna-mics	Winter Distribution	Diet	Biological Information	Seasonal Presence in Lehua Area	Citations[2]
Red-tailed tropicbird koa‘e ‘ula	660	87 g/day	Outside the breeding season, adults are solitary and pelagic	Forages on fish by diving into the water	Age at first breeding between 2 and 4 years; 1 egg/season; nests on ground; both parents incubate, brood, and feed	Breeding can occur throughout the year, but most nests active between February and June	Mitchell et al. 2005; VanderWerf et al. 2007
Glaucous-winged gull	1,180 (male) 950 (female)	Data not available	Farther from shore during winter; beaches and nearshore habitat, intertidal zone other seasons	Seizes small fish from near the water surface and forages for marine invertebrates	One of first species to first recolonize islands after removal of introduced mammalian predators; 2-3 eggs; highly territorial	Rare winter visitor to Lehua	Verbeek 1993; VanderWerf et al. 2007
Sooty Tern ’ewa’ewa	200 g	Data not available	Remain aloft outside of breeding season; pelagic	Squid, goatfish, flyingfish, mackerel scad	First breeding at 4 to 10 years; nests on shallow scrapes; 1 egg; high site fidelity; both parents incubate, brood, and feed chicks.	Reported recently as a rare visitor to Lehua; only breeds in large colonies between February and September	Mitchell et al. 2005; VanderWerf et al. 2007
Brown Noddy noio kōhā	180 g	Data not available	Remain near breeding grounds (within 62 miles) year-round	Fish and squid	First breeding at 3 to 7 years; 1 egg; nest on ground, cliffs, trees; both parents incubate, brood, and feed chicks.	Previously extirpated on Lehua; Only breeds in large dense colonies in spring and summer	Mitchell et al. 2005; VanderWerf et al. 2007
Black Noddy noio	108	29 g/day	Remain near breeding grounds (within 50 miles) year-round	Juvenile goatfish, lizardfish, herring, flyingfish, and gobies	First breeding at 2 to 3 years; Nests on ledges in back of sea caves; egg laying occurs year-round, although no nests found on Lehua in February; high site fidelity; 1 egg; both parents incubate, brood, and feed chicks.	Present year-round and presumable breeding in the sea caves	Mitchell et al. 2005; VanderWerf et al. 2007

[1] Mass values from Birds of North America, www.bna.ed

Table 5. Acute Toxicity of Diphacinone and Brodifacoum to Seabirds Wintering in the Lehua Area.[1,2]

Note: 1 pound = 454 grams	Amount of rodenticide that would have to be directly eaten to kill 50% of the population				Amount of contaminated prey that would have to be eaten to kill 50% of the population			
	Diphacinone		Brodifacoum		Diphacinone		Brodifacoum	
	mg of active ingredient	Grams of bait pellets (50 ppm)	mg of active ingredient	Grams of bait pellets (25 ppm)	Grams of Mussels	Grams of Fish Liver	Grams of Mussels	Grams of Fish Liver
Black-footed albatross chick	1,120	22,400	0.73	29.1	2,731,707	28,000,000	1,776	18,200
Laysan albatross chick	960	19,200	0.62	25.0	2,341,463	24,000,000	1,522	15,600
Brown booby	536	10,720	0.35	13.9	1,307,317	13,400,000	849.8	8,710
Red-footed booby	400	8,000	0.26	10.4	975,610	10,000,000	634	6,500
Black noddy	43	860	0.03	1.1	105,366	1,080,000	68.5	702
White-tailed tropicbird	182	3,640	0.12	4.73	443,902	4,550,000	288.5	2,958
Red-tailed tropicbird	264	5,280	0.17	6.9	643,902	6,600,000	418.5	4,290
Great frigatebird	540	10,800	0.35	14.0	1,317,073	13,500,000	856.1	8,775
Glaucous-winged gull	380	7,600	0.25	9.9	926,829	9,500,000	602.4	6,175
Sooty tern	80	1,600	0.05	2.1	195,122	2,000,000	127	1,300
Brown noddy	72	1,440	0.05	1.9	175,610	1,800,000	114.1	1,170

1 Based on the lower of the two acute oral LD_{50} values for bobwhites or mallards (>400 mg/kg body weight for diphacinone, 0.26 mg/kg body weight for brodifacoum).

2 Based on the maximum tissue residue in mussels and fish liver recorded

3.4.2 Potential Impacts to Migratory Shorebirds Present on Lehua in the Winter

Biology and Status

Two species of shorebirds are present on Lehua during the winter: the Pacific golden-plover and the ruddy turnstone. Neither species nests in Hawai‘i. Both species are present in small numbers on Lehua during the winter. Six golden-plovers and 9 ruddy turnstones were observed during a recent winter expedition to Lehua (VanderWerf et al. 2007). The ruddy turnstone feeds on marine invertebrates in the intertidal zone. The golden-plover feeds on terrestrial insects and intertidal invertebrates (Table 6). Other shorebird species, such as wandering tattlers and sanderlings, are common in Hawai‘i in the winter but have not been observed on Lehua.

Potential Impacts from Direct Ingestion of Rodenticide (Primary Nontarget Hazard)

Ruddy turnstone and Pacific golden-plover, which both forage in intertidal areas (see Table 6), are likely to be present during the winter operational window on Lehua and could potentially be exposed to rodenticide. Although pellets could be available in the intertidal area, it is highly unlikely that these species would actually forage on bait pellets given their normal feeding behavior, the low density of pellets, and the small number of shorebirds on Lehua.

Even if a bird were to pick up diphacinone bait pellets, the ruddy turnstone would have to consume approximately 640 g (almost 1.5 pounds) and the Pacific golden-plover would have to consume approximately 1,200 g (almost 2.7 pounds) of diphacinone bait to deliver an LD_{50}–equivalent dosage (based upon the lower reported acute oral LD_{50} of >400 mg/kg body weight for bobwhites). It would be physically impossible for either species to consume that much bait in one or several days. However, the projected LOEL (extrapolated from the lowest reported LOEL for diphacinone in birds, 0.11 mg/kg/day, Saverie et al. 1977) of diphacinone for a ruddy turnstone is 0.01 mg/day or about 0.2 gram of bait per day and for a Pacific golden-plover it is 0.02 mg/day or about 0.3 gram of bait per day (Table 6). As long as bait is present in a treated area, such a level of non-lethal exposure would be possible. However, the bird would most likely not consume it based on feeding habits.

Based on the acute oral LD_{50} figure reported for mallards (0.26 mg/kg body weight, Table 6), a ruddy turnstone would only have to consume 0.8 g of a 25 ppm brodifacoum bait, or about 50% of one average-sized pellet, to obtain an LD_{50}–equivalent dosage; while a Pacific golden-plover would only have to consume 1.6 g of a 25 ppm brodifacoum bait, or about one average sized pellet, to obtain an LD_{50}–equivalent dosage (Table 6). The lethal effects of brodifacoum have been confirmed in northern New Zealand dotterels (*Charadrius obscurus acquilonius*), and observed in an additional two shorebird species on a mainland mammal eradication project (pied stilts, *Himantopus himantopus;* and spur-winged plovers, *Vanellus miles nova novaehollandiae*) (Dowding et al. 1999, Dowding et al. 2006). Again, no LOEL has been determined for brodifacoum because of its substantially higher toxicity all doses administered have had measurable effects.

In conclusion, the potential is very low for any direct adverse impacts to shorebirds from directly consuming either diphacinone or brodifacoum pellets, since neither species is likely to feed directly on pellets, pellets will be distributed at very low densities, and few shorebirds use Lehua. Even if they did feed on diphacinone pellets, it would be physically impossible for either species to consume a lethal dose.

Table 6. Biological Characteristics of Shorebirds Present on Lehua Island in the Winter

Species[1]	Mass[1] (g)	Energy Dynamics	Winter Distribution	Diet	Biological Information	Seasonal Presence in Lehua Area	Citations[2]
Pacific golden-plover Kōlea	150	No information	Common on all main Hawaiian Islands (August-April) along shorelines and grassy areas	Terrestrial insects and intertidal marine invertebrates	High site fidelity to wintering grounds and territories within those areas in Hawai‘i; no nesting	Winter only	Mitchell et al. 2005; VanderWerf et al. 2007
Ruddy turnstone ‘akekeke	80	No information	Common on all main Hawaiian Islands (August-April). Found on rocky shorelines with abundant seaweed and on mudflats	Primarily marine invertebrates, including worms, small fish, bivalves and crustaceans	Age of first breeding is 2 years; 3-4 eggs/clutch; nests on ground in tundra; both parents incubate and feed young; 1 clutch per year	Winter only	Mitchell et al. 2005; VanderWerf et al. 2007

[1] Mass values from Birds of North America, www.bna.edu

Potential Impacts from Indirect Ingestion of Rodenticide (Secondary Nontarget Hazard)

A ruddy turnstone would have to consume over 78,049 g (172 pounds) of mussels with diphacinone in their tissues to obtain the equivalent LD_{50} dose, which is physically impossible. For brodifacoum, a turnstone would have to eat 50.7 g (1.8 ounces) of contaminated mussels, which is unlikely. The LOEL for secondary hazard for diphacinone would be 21 g of contaminated mussels and 220 g of fish liver. Only if contaminated tissue were available over several days would there be any risk of obtaining an LOEL for the turnstone through secondary exposure to diphacinone. This is unlikely because of the small amount of bait to which marine invertebrates might be exposed in the intertidal zone, with no more than four applications of diphacinone (and more likely two), each five to seven days apart. In the unlikely event that brodifacoum is used, it would probably only be applied once. The ruddy turnstone would not be adversely impacted with diphacinone because of the impossibly large amount of contaminated invertebrates that would need to be consumed, nor with brodifacoum, because it is unlikely there would be enough invertebrates exposed to the degree necessary to accumulate significant levels of toxins.

The Pacific golden-plover would have to consume over 146,341 g (323 pounds) of mussels with diphacinone in their tissues to obtain the equivalent LD_{50} dose, which is physically impossible. For brodifacoum, the level is 95.1 g (3.4 ounces) of contaminated mussels, which is unlikely. The LOEL for secondary hazard would be 40 g of mussels contaminated with diphacinone and 413 g of fish liver. Only if contaminated tissue were available over several days would there be any risk of obtaining an LOEL for the Pacific golden-plover through secondary exposure to diphacinone (Table 6).

In conclusion, the potential is very low for any indirect adverse impacts to shorebirds from consuming prey items contaminated with either diphacinone or brodifacoum, primarily because intertidal organisms are not expected to accumulate rodenticides in their tissues. Even if shorebirds did feed on contaminated prey, it would be physically impossible for them to consume a lethal dose of diphacinone. It is physically possible but unlikely in this context for shorebirds to consume a lethal dose of brodifacoum in prey tissue, given the low probability that invertebrates will be exposed to enough rodenticides to accumulate it in their tissues.

Table 7. Acute Toxicity of Diphacinone and Brodifacoum to Shorebirds Wintering in the Lehua Area.[1,2]

Note: 1 pound = 454 grams	Amount of rodenticide that would have to be directly eaten to kill 50% of the population				Amount of contaminated prey that would have to be eaten to kill 50% of the population			
	Diphacinone		Brodifacoum		Diphacinone		Brodifacoum	
	mg of active ingredient	Grams of Bait (50 ppm)	mg of active ingredient	Grams of Bait (25 ppm)	Grams of Mussels	Grams of Fish Liver	Grams of Mussels	Grams of Fish Liver
Pacific golden-plover	60	1,200	0.04	1.6	146,341	1,500,000	95.1	975
Ruddy turnstone	32	640	0.02	0.8	78,049	800,000	50.7	520

1. Based on the lower of the two acute oral LD_{50} values for bobwhites or mallards (>400 mg/kg body weight for diphacinone, 0.26 mg/kg body weight for brodifacoum)
2. Based on the maximum tissue residue in mussels and fish liver recorded

3.4.3 Potential Impacts to Barn Owls, Cattle Egrets and Peregrine Falcons

Biology and Status

The barn owl, not native to Hawai‘i but native to North America, has been recently recorded on Lehua and could potentially visit the island in the winter. No breeding has been documented on Lehua. A sediment deposit beneath a roost on the southern shore of Lehua contained thousands of bones from Polynesian rats, feral rabbits, Bulwer’s petrels, brown noddies, zebra doves and several other bird species. One owl pellet contained the entire skull of a wedge-tailed shearwater, demonstrating that the owls prey on relatively large species (VanderWerf et al. 2007). Because barn owls eat rodents, it is possible that they could secondarily ingest rodenticide in poisoned rats.

The peregrine falcon is an extremely rare winter visitor from either Asia or North America, where it has been delisted under the Endangered Species Act. Single birds have been observed infrequently during winter months flying near Lehua but not landing (VanderWerf et al. 2007). Peregrine falcons feed primarily on small birds on the wing, so they would not be expected to scavenge bait pellets or feed on live or dead rodents. Because there is no likely pathway for poisoning for falcons, they will not be considered further.

Cattle egrets are not native, and some commute to Lehua from nearby Ni‘ihau and Kaua‘i. Adults are present in February but don’t nest until later spring and summer on Lehua. They may be predators on seabird eggs and chicks (VanderWerf et al. 2007) and appear to prefer live prey, although they are not known to eat live rats. They also would not be expected to scavenge bait pellets or eat dead rodents. Because there is no likely pathway for poisoning for egrets, they will not be considered further.

Potential Impacts from Direct Ingestion of Rodenticide (Primary Nontarget Hazard)

Barn owls only capture live prey and therefore would not ingest grain-based pellets (Table 8). Therefore, there is no potential for the barn owl to ingest rodenticide directly.

Potential Impacts from Indirect Ingestion of Rodenticide (Secondary Nontarget Hazard)

Because barn owls hunt live prey, they could eat live rats carrying rodenticide residues in their tissues prior to dying. The most conservative (worst case) analyses of these situations will be examined here, using data from the literature. To assess secondary nontarget hazards for the barn owl, the analysis will use whole body values with the maximum residue levels documented in rodents (Erickson and Urban 2004). The LD_{50} for an average sized 315 g (0.7 lbs) owl is 0.1 mg of brodifacoum and 126 mg of diphacinone. To ingest these amounts of rodenticides secondarily via rodents contaminated to the highest level documented, an owl would need to consume 3.15 g (0.1 ounce) of a brodifacoum-loaded rat or 37 kg (81.6 pounds) of a diphacinone-loaded rat. An owl could obtain an LOEL dosage of diphacinone by eating 10 g of these contaminated rodents (Table 9). Even under these extreme situations, the risk of mortality due to using a diphacinone formulation is essentially zero.

Table 8. Biological Characteristics of Barn Owls Present in Winter on Lehua Island

Species	Mass[1] (g)	Energy Dynamics	Winter Habitat	Diet	Biological Information	Seasonal Presence in Lehua area	Citations[1]
Barn owl	378 (female) 315 (male)	41 g to maintain weight for 24 hours (1-2 adult voles/day)	Open or semi-open country	Live rats and small birds, including seabirds, on Lehua	3-8, sometimes 12 or more eggs/clutch, 1-2 broods per year	Year-round resident, probably flies in from Ni`ihau and Kaua`i	Kaufmann 1996

[1] Mass values from Birds of North America, www.bna.edu

Table 9. Acute Toxicity of Diphacinone and Brodifacoum to Barn Owls Present in Winter on Lehua Island

***Note*: 1 pound = 454 grams**	**Amount of rodenticide that would have to be directly eaten to kill 50% of the population**				**Amount of contaminated prey that would have to be eaten to kill 50% of the population**	
	Diphacinone (50 ppm)		**Brodifacoum (25 ppm)**		**Diphacinone**	**Brodifacoum**
	mg of active ingredient	**Grams of Bait**	**mg of active ingredient**	**Grams of Bait**	**Grams of Rodents[1]**	**Grams of Rodents[1]**
Barn owl (315 g body mass)	126	2,520	0.08	3.30	37,059	3.15

[1] Based on maximum whole body residues recorded in rodents: 3.4 ppm diphacinone, 25.97 ppm brodifacoum.

Using a brodifacoum product, however, could create a substantial risk to individual barn owls on Lehua. However, brodifacoum would only be used if diphacinone fails and it can be shown that eradication failed due to the use of diphacinone rodenticide, not other factors. Because this scenario is unlikely, there is little risk from the proposed project to nonnative barn owls. However, in the event that a barn owl died as a result of ingesting brodifacoum, it would not affect the population significantly since barn owls are visitors from the adjacent islands such as Kaua‘i and Ni‘ihau (VanderWerf et al 2007), where the large owl populations would not be affected and could rapidly provide additional birds.

3.5 Potential Impacts to Monk Seals

Potential impacts to monk seals were discussed in the 2005 EA and in the 2005 informal section 7 consultation with NMFS. Monk seal use of Lehua does not vary seasonally so switching to a winter operational season will not change anything with regard to the 2005 impact analysis. None of the other proposed modification will increase risk to monk seals. NMFS confirmed this in April 2008 when they stated that it would not be necessary to re-initiate section 7 consultations for this project, as modified. In short, there is no probable pathway of injury since monk seals are not likely to eat bait pellets and there is only a slight risk that marine organisms eaten by monk seals could become contaminated. As stated in the 2005 EA, helicopters will not fly directly over or apply rodenticides onto monk seals hauled out on Lehua. Project personnel on island will also try to maintain a 100’ distance from hauled out seals. For all these reasons, no impacts are anticipated.

3.6 Consistency with Hawai‘i State Enforceable Policies per CZMA, Federal Endangered Species Act, National Historic Preservation Act, and Clean Water Act

3.6.1 Consistency with Applicable State Coastal Management Policies

The following objectives and policies of HRS 205A-2 (Coastal Zone Management) would apply to the proposed project (J. Nakagawa, Hawai‘i Coastal Zone Management Program, Hawai‘i Office of State Planning, pers. comm.), with evaluation of consistency:

- (b)(4)(A) Protect valuable coastal ecosystems, including reefs, from disruption and minimize adverse impacts on all coastal ecosystems.
    - **Consistency rationale:** The native ecosystems on Lehua have been disrupted by invasive rats. This project intends to eradicate the rats to allow the plant and seabird components of the ecosystems to recover naturally when possible and to provide the foundation for actively removing invasive weeds for supporting the restoration of native plant communities. These actions are consistent with the purposes of HAR 13-125 regarding State Wildlife Sanctuaries. No adverse impact will occur to any marine vertebrate or invertebrate communities and species, nor to marine plant communities.

- (c)(4)(C) Preserve valuable coastal ecosystems, including reefs, of significant biological or economic importance.
    - **Consistency rationale:** Rats are an ongoing threat to native plants and animals on Lehua and eradication will benefit native species. Lehua has remnant populations of native plant species that will be preserved with the rat eradication project. Existing seabird species will have the potential to recover to larger populations if rats are removed, and species that are not found on Lehua but found on adjacent islands may be able to recolonize available habitat. Again, no adverse impact will occur to any marine vertebrate or invertebrate communities and species, or to marine plant communities.
- (c)(4)(E) Promote water quantity and quality planning and management practices that reflect the tolerance of fresh water and marine ecosystems and maintain and enhance water quality through the development and implementation of point and nonpoint source water pollution control measures.
    - **Consistency rationale:** Water quality will not be adversely impacted because:
        - No surface water is found on Lehua;
        - Extremely small amounts of rodenticide will enter the marine environment when applied as described in Chapter 2;
        - The rodenticide pellets that do enter the marine environment break up rapidly in the intertidal dynamics;
        - Studies made of a huge point source spill of brodifacoum in New Zealand indicate that marine invertebrates are not adversely affected; the minute amounts of diphacinone entering the marine environment would have no adverse impacts to water quality.
        - No diphacinone residues were detected in any seawater samples collected at Mokapu Island after the February 2008 aerial rodenticide broadcast.

3.6.2 Consistency with State Enforceable Policies

The following four State laws and associated regulations, as well as their Federal counterparts, are described in detail in Chapter 1. Consistency with these state enforceable policies are evaluated for each law and found consistent.

HRS 149A: Hawai‘i Pesticides Law and FIFRA

Consistency rationale: Both diphacinone and brodifacoum are “restricted use” pesticides. The USDA will obtain the necessary permits from the State Department of Agriculture for aerial application of the rodenticide and all rodenticide application will be under the direct supervision of a certified applicator. Per both FIFRA and HRS 149A, all application will be according to the label, and no pesticide will be used that does not have an approved label.

HRS 195D and HAR 13-124: Conservation of Aquatic Life, Wildlife, and Land Plants (Endangered Species) and Federal Endangered Species Act

Consistency rationale: No threatened or endangered bird species are known to be present on Lehua in the winter but the endangered Hawaiian monk seal could be present. No listed plants or insects are present.

Intra-Service Section 7 Endangered Species Act Consultation for the Newell's shearwater and Hawaiian petrel (listed), and the band-rumped storm-petrel (candidate) was finalized in April 2005 and included in the 2005 final EA for the Lehua Island project. The USFWS determined that the proposed action would have positive effects on the ecosystem and the three species of seabirds, resulting in a determination of "may affect but is not likely to adversely affect" the shearwater and petrel, and a determination of "no effect" on the storm-petrel. With the change to a winter operation, when listed seabirds are not present, no impact is anticipated. All operations would be conducted during the day.

Informal Section 7 consultation with the National Marine Fisheries Service (letter dated July 5, 2005, Appendix E of the 2005 EA) also determined that the proposed eradication project on Lehua Island was not likely to adversely affect federally listed Hawaiian monk seals or sea turtles. The letter also documented that the USFWS found that "bait pellets will not present a poisoning hazard to foraging seals or sea turtles." NMFS concurred with this finding and further stated: "It should also be noted that as a result of this project there could be indirect beneficial effects to both monk seals and sea turtles arising from increased native plant cover which will stabilize soils, reduce sediment runoff into the ocean and improve marine water quality. This may result in the establishment of improved nearshore foraging habitat for both monk seals and sea turtles. Given the mitigation put in place under the draft EA we conclude that any effects of the proposed action on monk seals or sea turtles would be discountable. NOAA Fisheries Service therefore concurs with your determination that the project may affect but is not likely to adversely affect ESA listed species under our jurisdiction." When contacted again by USFWS in April 2008 and asked if the proposed project modifications would require re-opening of the informal consultation, NMFS replied: "Although the proposed action has been slightly modified (application season changed from summer to winter, deflector no longer will be used for application), these modifications do not change the effects of the proposed action to ESA-listed marine species. Thus the 2005 concurrence letter is still valid, and there is no need to reinitiate Section 7 consultation (email from L. Smith, ESA Section 7 Coordinator, Honolulu, email dated April 14, 2008).

Therefore, the informal Section 7 consultations conducted with the USFWS and NMFS fulfills compliance with both state and federal law and regulations.

HRS Chapter 6E: Historic Preservation and Federal National Historic Preservation Act

Consistency rationale: Lehua has several historical sites, one of which has been data-recovered and all the others marked with tags. Since bait will be applied from the air, bait application will not adversely affect these sites. Placing pre-operational rat and bait monitoring gear, as well as conducting post-operational monitoring, will require limited foot traffic. All personnel will be trained to avoid disturbing these sites, which have all been marked by a qualified archaeologist. No digging or other excavations will be

conducted during operations or monitoring. No cultural practices are currently known to occur on Lehua Island itself. Subsistence gathering in waters around Lehua rarely if ever occurs in the winter months and therefore is not expected to be impacted. Rodenticide residues are not expected to accumulate in subsistence species. Therefore, no impact would occur to cultural structures and practices. The State Historic Preservation Officer has concurred with the USFWS determination of "No Adverse Effect" on significant historic sites on Lehua Island from the project (letter dated 10/17/05), as long as the following mitigation measures are completed: 1) Submission of a completed archaeological inventory survey report; 2) Recovery of data from a hearth site by a qualified archaeologist; and 3) placement of site tags on historic properties prior to restoration. All mitigation measures will be completed prior to initiating rodent eradication.

HRS 342D and HAR 11-54 Water Pollution and Water Quality Standards; HAR 11-55 and Federal Clean Water Act

Consistency rationale: Per HAR 11-54-4(b)(3), no rodenticide, including diphacinone and brodifacoum, is identified as a toxic pollutant. No disturbance of soil and no construction activities are included in the proposed action.

The minute amount of rodenticide pellets that might enter nearshore marine waters would disintegrate quickly and be dispersed. Therefore, the pellets and the active ingredient would not:

- form either a bottom sludge nor floating materials;
- change any water characteristics;
- be toxic to any marine life;
- encourage any nonnative marine life.

Consistency rationale: HAR 11-54-6 (b) defines the waters around Niihau and Lehua as Class AA open coastal waters and sets numerical water quality parameters that must not be exceeded in such areas, including criteria for total nitrogen, ammonia nitrogen, nitrate+nitrite nitrogen, total phosphorus, light extinction coefficient, chlorophyll and turbidity. However, use of diphacinone or brodifacoum rodenticides could not result in exceedances of these parameters because:

- Rodenticides contain little or none of these chemical compounds; and
- The minute amount of rodenticide pellets that might enter nearshore marine waters would disintegrate quickly and be dispersed and therefore would not cause turbidity or light extinction.

Consistency rationale: No NPDES permit is required under either the Federal Clean Water Act per 40 CFF 122.3 or per State of Hawai‘i HAR 11-55-04(h), as explained previously.

Consistency rationale: Environmental sampling following a similar Hawai`i project did not detect any diphacinone residues in the environment. Seawater, limpets and fish were sampled around Mokapu Island, Moloka`i following two aerial applications of diphacinone to eradicate rats in February 2008. Two independent laboratories tested the

samples, with detection limits set in the low parts per billion range, and neither detected any trace of diphacinone. This indicates that even if diphacinone pellets did enter the water, they did not leave detectable residues in water or marine tissues.

3.7 Cumulative Impact Analysis

Under the National Environmental Policy Act (NEPA), cumulative effects are defined as:

> "The impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time." (40 CFR 1508).

Under Endangered Species Act (ESA) regulations cumulative effects are defined as:

> "Those effects of future State or private activities, not involving Federal activities, that are reasonably certain to occur within the action area of the Federal Action subject to consultation." (50 CFR 402.2)

The U.S. Fish and Wildlife Service further defines "State or private activities" as including tribal, local, or private actions that are reasonably certain to occur in the action area considered. Future Federal actions that are unrelated to the proposed action are not considered because they require separate evaluation under Section 7 consultation. The past and present impacts of non-Federal actions are part of the environmental baseline. The lighthouse managed by the Coast Guard does not adversely impact any resources on Lehua and no additional actions were identified in the 2005 EA.

Overall, because the proposed rat eradication project is under the jurisdiction of DOFAW (the island is a State Wildlife Sanctuary), no further cumulative impacts would occur to the species evaluated below under either NEPA or the ESA beyond those already having occurred or continuing to occur under the baseline (i.e, under the no action alternative as described and analyzed in the 2005 EA). No other non-Federal action could occur on the island without full approval of DOFAW. No planned actions or even proposed actions other than this ecological restoration project are foreseen at this time. Therefore, foreseeable actions will have no contributory adverse impacts to any resources evaluated in this supplement.

Even with four applications of diphacinone in the winter of 2008 to 2009, no long-term cumulative impacts are expected for any species or resource, as evaluated in this chapter. Again, although the hazards to nontarget birds are substantially higher with brodifacoum than diphacinone, the analyses in this chapter indicate that no long term adverse cumulative effects are foreseen with brodifacoum, even if potentially impacted alien bird populations are reduced. It is expected that population recovery would take longer with brodifacoum than with diphacinone, but that it would occur, especially with ingress from alien bird populations on Kaua‘i and Ni‘ihau. If quarantine fails in the future and rats re-invade the island, then the proposed action may be repeated. This is not expected to occur and, even if it does, it would not occur for at least two years. Therefore, any

impacted populations would be expected to have recovered and no cumulative impacts would occur to those populations.

3.8 State Evaluation of Significance of Impacts per HRS 343

The State of Hawai‘i Environmental Council gives 13 criteria (in italics below) for defining significant project impacts (Hawai‘i Administrative Rules, Section 11-200-12). As discussed below, this project does not trigger any of the criteria for significance and thus, under State law, does not require preparation of an environmental impact statement (EIS). Federal criteria at 40 CFR 1508.27(b) for significance and the State criteria for significance listed below are similar but not identical.

The proposed actions do not involve an irrevocable commitment to loss or destruction of any natural or cultural resource. The actions will contribute to the restoration of a healthy native ecosystem on Lehua by eradicating nonnative rats (Chapter 1). These actions are also consistent with the Hawai‘i Comprehensive Wildlife Conservation Plan (Mitchell et al. 2005).

The proposed actions will not curtail the range of beneficial uses of the environment. The activities proposed are intended to contribute to ecological restoration of the island and improve habitat for the native plants and nesting seabirds that inhabit or historically inhabited the island, prior to its degradation by invasive rats. Restoration of Lehua will thus improve the range of beneficial uses of the environment, including for endangered seabirds, Hawaiian monk seal and sea turtles (Chapter 1).

The proposed actions will not conflict with the State's long-term environmental policies. The proposed actions will not conflict with the environmental policies set forth in HRS Chapter 344 and the State written policies and enforceable policies (Chapter 3) and other statutes and regulations, since the proposed actions will not damage sensitive natural resources. Instead, they will improve the environment of Lehua (Chapter 1).

The proposed actions will not substantially adversely affect the economic and social welfare of the community. The proposed activities utilize the most effective strategies to eradicate invasive rats as well as mitigating potential adverse impacts, thus contributing to the restoration of the ecosystem of Lehua. With ecosystem restoration, seabird populations will most likely increase and additional species will most likely return to Lehua, increasing its value as a State Seabird Sanctuary. Therefore, the proposed project will result in an improved environment, thus supporting eco-tourism and enhancing economic and social welfare (Chapter 1).

The proposed actions will not substantially adversely affect the public health of the community. The rodenticides have been found to have no adverse impacts on water quality or on marine life that might be consumed by people (Chapter 3).

The proposed actions will not involve substantial secondary impacts, such as population changes or effects on public facilities. Lehua is a small island designated as a State Seabird Sanctuary and is uninhabited and undeveloped. The project does not propose construction of public facilities or involve establishing a human population. Thus, the proposed actions will not affect any public recreational facilities and will not induce population growth or decline in the area.

The proposed actions will not involve a substantial degradation of environmental quality. Modifying the project to be conducted in the winter and using diphacinone as the primary rodenticide will minimize impacts to the environment during the implementation of the proposed actions. No species listed under the ESA, except potentially the endangered Hawaiian monk seal, will be present during the winter, and NMFS has identified mitigation regarding protecting monk seals during operations. Restoration will increase the environmental quality of the ecosystems of Lehua for its flora and fauna (Chapter 3).

The proposed actions will not affect a rare, threatened or endangered species or its habitat. The operation as modified will benefit native plant and animal species protected under the Federal and state endangered species laws. The limited and temporary human activities associated with the modified operation will have a negligible impact on listed species because most will not be present during the winter and conservation actions identified during the informal Section 7 consultation will be implemented for the monk seal (Chapters 1 and 2).

The proposed actions will not have cumulative impacts or involve a commitment for larger actions. The analyses show that the modified operation and mitigation measures integrated into the proposed actions, such as the use of diphacinone and conducting operations during the winter when presence of nontarget and listed species is minimal, will result in no cumulative impacts. No other known or potential actions would contribute to or cause any cumulative impacts (Chapter 3).

The proposed actions will not substantially affect air or water quality or ambient noise levels. The proposed actions are fully consistent with both Federal and State water quality laws and regulations. Helicopters will cause noise for a period of up to four non-consecutive days during aerial application of rodenticides on Lehua, but the effect will be highly temporary and no people not associated with the operation will be present on the island (Chapter 2).

The proposed project is not located in an environmentally sensitive area (e.g. flood plain, tsunami zone and coastal zone). Although the site is located in a State Seabird Sanctuary, the proposed actions are in accordance with HAR 13-125, as well as Federal and State Coastal Zone Management policies and enforceable policies. All actions will protect sensitive resources, including the coastal zone while meeting ecological management objectives. Project actions are in accord with environmental management goals of USFWS and DOFAW (Chapter 1).

The proposed actions will not substantially affect scenic vistas and view planes identified or State plans or studies. The project does not involve construction of any permanent structures or alteration of landscapes. Thus, it will not affect any sites or vistas.

The proposed project will not require substantial energy consumption. The affected area is not on a local power grid. The only energy uses will be using motorized vehicles for accessing points of departure to the island and for broadcasting bait via helicopter for up to 4 days total over several months. All work will be conducted during daylight hours.

4.0 LIST OF PREPARERS

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APPENDIX A

INTRODUCTION TO RODENTICIDES AND RODENTICIDE HAZARD ANALYSIS, WITH SPECIAL REFERENCE TO BIRDS

Both diphacinone and brodifacoum are chronic rodenticides, meaning that the onset of symptoms only begins sometime after the lethal dosage has been ingested. If a rat does not experience symptoms until long after ingesting a lethal dose of the rodenticide, it can not associate the symptoms with the new food item, causing the rats to continue eating the bait until or even long after a lethal dose has been ingested.

Diphacinone and brodifacoum are anticoagulants which act by disrupting the normal blood-clotting mechanisms of vertebrates by competing with vitamin-K, a chemical necessary for clotting of blood, for receptor sites in the liver. Death in animals receiving a lethal dose of an anticoagulant rodenticide typically occurs from shock due to excessive blood loss through internal and sometimes external hemorrhaging eventually causing severe anemia. Prior to dying, between the time of ingestion and actual death (latent period), poisoned animals may exhibit increasing weakness and behavioral changes such as acting sluggish, changes in activity time, and reduced predator avoidance ability. This behavior can make target rodents more susceptible to predation (Cox and Smith 1990, Newton et al. 1990, Innes and Barker 1999).

Anticoagulant rodenticides are divided into two chemical groups, the indandiones, such as diphacinone and the coumarins; which includes brodifacoum. More informally, anticoagulant rodenticides are also described either as "first generation" or "second generation" rodenticides, simply referring to the time period during which they were developed. Diphacinone is a first generation and brodifacoum a second generation rodenticide. Second generation compounds were specifically designed to overcome resistance to warfarin (an early "first generation" compound) and are therefore generally more toxic than the first generation rodenticides. The coumarins in general, but especially brodifacoum, are characterized by an increased potential for accumulation and persistence in body tissues. This is due primarily to their greater affinity to bind to receptors in the liver and the long latent period during which rodents continue to feed on the toxicant (Eason and Wickstrom 2001, Fisher et al. 2003).

Comparison of Brodifacoum and Diphacinone Characteristics

Brodifacoum is more toxic than diphacinone and is retained much longer in the body tissues of exposed animals, especially the liver, than diphacinone. Animals may ingest a lethal dose of brodifacoum more quickly than with diphacinone; however, death is still typically delayed from 4 days to about 2 weeks for both rodenticides. During this extended latent period between ingestion of the lethal dose and death, the animals continue to feed on the brodifacoum bait and build up ever higher levels of toxic residues in their tissues. In contrast, diphacinone, because it is less toxic and more rapidly metabolized and excreted, accumulates in body tissues less readily and in lower concentrations (Erickson and Urban 2004).

Products containing diphacinone were first registered for rodent control in 1960 at active ingredient concentrations of 0.005% to 0.01 % (50 to 100 ppm). Diphacinone (0.005% active ingredient) is currently registered for use for conservation purposes in the United States. Brodifacoum was first registered for rodent control in and around buildings in 1979 and is now registered for conservation purposes in the United States.

Many laboratory studies of the LD_{50} for vertebrate species have been conducted on a variety of test species (both target and nontarget species) using a range of methods (Swift 1998, Fisher 2005). In general, the median oral lethal dosage of diphacinone for rats is about 3.0 mg/kg, while for brodifacoum it is roughly 0.3 mg/kg. Brodifacoum is about ten times more toxic on a weight/weight basis to rats than diphacinone. However, as previously mentioned, there is a similar latent period between the time of ingestion and death between the two toxicants. Many factors influence this delay, but in general the latent period is about seven days and ranges from three to 14 days for both of these rodenticides (Eason and Wickstrom 2001, Erickson and Urban 2004).

A rodenticide that is rapidly metabolized and/or excreted from the primary consumer (the animal directly ingesting the rodenticide) poses fewer hazards to secondary consumers than one that is readily retained in tissues and therefore accumulates in the bodies of animals over time. Sublethal exposure to anticoagulants can produce significant blood clotting abnormalities and internal and external hemorrhaging. Such chronic hemorrhaging might be especially detrimental if combined with other factors such as adverse weather, food shortages, pregnancy or predation stressors, and could predispose an animal to death from other sources, such as bruising, food stress, and reduced potential for recovery from wounds and accidents.

Most rodents will continue eating for several days or more after ingesting a lethal dose of an anticoagulant rodenticide. In a laboratory study with wild caught brown rats the average number of LD_{50} doses of brodifacoum (50 ppm bait) ingested was 80 if feeding only on bait, and as many as 40 LD_{50} doses were ingested prior to dying if offered a choice of bait or nontoxic food (after ICI Americas, Inc. 1978b, cited in Erickson and Urban 2004). Another similar laboratory study found that rats (*Rattus norvegicus* Wistar) in an *ad libitum* 2-choice study ate almost 25 LD_{50} doses of a brodifacoum (20 ppm) bait formulation resulting in liver residues of 10.7 mg/g (Fisher et al. 2004). For comparison, Brodifacoum-25D is 0.0025% (25 ppm) a.i. or 2.5 mg/g of bait. Therefore, the livers of these rats contained more than four times the active ingredient concentration of the actual brodifacoum bait formulation.

Using the same procedures, the same study found that rats ate over twelve LD_{50} doses of a diphacinone bait formulation resulting in liver residues of 4.7 mg/g. For comparison, Diphacinone--50 is 0.005% a.i. or 5 mg/g (Fisher et al. 2004). Therefore, the livers of these rats actually contained slightly less than the active ingredient concentration of the actual bait formulation.

Generally, repeated exposures to small doses of anticoagulants over several days pose a greater hazard than larger single doses. Anticoagulants bind to receptors in the liver and other tissues, including the kidneys, pancreas, lungs, brain, fat and muscles and are eliminated from the liver last. The length of time a rodenticide is retained in tissues or

how quickly it is eliminated (half-life) greatly influences accumulation of rodenticides in tissues and, therefore, nontarget hazards.

Elimination of anticoagulant rodenticides from tissues is biphasic, with a proportion of the toxicant excreted within a shorter time and the remainder bound in the tissues and excreted over a much longer period of time (Parmer et al. 1987, cited in Fisher et al. 2003). The first phase of brodifacoum excretion from tissues takes about 60 days, with the second phase lasting almost 300 days. In contrast, 70% of a single dose of diphacinone may be excreted in about 8 days. In a laboratory test, 0.1 mg/kg of brodifacoum was administered to rats, resulting in mean liver residue concentrations of 1.27 mg/kg at one week, 0.59 mg/kg at 18 weeks and 0.49mg/kg at 24 weeks. The study estimated the liver elimination half-life of brodifacoum to be 113.5 days. In the same test, 0.8 mg/kg of diphacinone was administered to rats, resulting in mean liver residue concentrations of 0.08 mg/kg at one week and below the detectable limit at six weeks. Further trials of diphacinone resulted in the estimated liver elimination half-life 3 days (Fisher et al. 2003). In addition, the range of whole carcass residues reported by the EPA in primary consumers was 2.07 to 25.97 ppm for brodifacoum and 0.48 to 3.4 ppm for diphacinone.

Therefore, brodifacoum presents a substantially higher potential for causing secondary exposure to predators and scavengers than diphacinone.

Efficacy Studies of Brodifacoum and Diphacinone

The following information is compiled from Erickson and Urban (2004) and the New Zealand Pesticide Toxicology Manual (New Zealand Department of Conservation 2001).

Brodifacoum has been used for most rat eradication projects worldwide because its far greater toxicity is perceived to impart a greater probability of success. However, it is important to remember that toxicity and efficacy are not synonymous terms. Efficacy is a complex interaction of many factors, including bait acceptance, application rate, application method, toxicity, and timing of application when rodent populations, reproduction and alternate foods are lowest to ensure eradication. The eradication of rodents on islands has been successfully implemented using the generally less toxic anticoagulant rodenticides warfarin, pindone, diphacinone and bromadiolone (Witmer et al. 2001, Donlan et al. 2002, Dunlevy and Scharf 2008) and some eradication efforts have failed during operations using brodifacoum (Tyrell et al. 2000, Clout and Russell 2006, Howald et al. 2006).

Recently, however, an increasing number of experts in island rodent eradication and control have recommended using less toxic rodenticides such as diphacinone, and decreasing the use of more persistent and toxic rodenticides such as brodifacoum on future projects because of the greater risk to nontarget species associated with brodifacoum, including both primary hazards (when nontarget species feed directly on the bait) and secondary hazards (when nontarget species feed on rodenticide-exposed animals with rodenticide residues in their tissues) (Tobin 1994, Eason et al. 1999, Fisher et al. 2003). New Zealand has a policy of reducing brodifacoum use on mainland sites, but still only uses brodifacoum in offshore island eradications (Hoare and Hare 2006). Fisher et al. (2004), recommend conducting additional field studies using diphacinone to

further determine efficacy and validate estimates of lower risk for secondary poisoning of nontarget species.

A number of laboratory and field studies in the United States have evaluated the effectiveness of various application methods and the efficacy of diphacinone for control of rat populations, especially in Hawai‘i:

- Laboratory trials using Sprague-Dawley strain laboratory rats found that 100% of 20 laboratory-bred brown rats died after consuming an average of 42 grams of bait (0.21 g of the a.i. diphacinone), 7 g per day per animal over an average of six days (Svircev 1992).
- Laboratory trials found that 100% of 20 Hawaiian wild-caught Polynesian rats died over two to ten days after consuming an average of 19.7 grams of bait (0.099 g of 0.005% diphacinone) per animal and 95% of 20 wild-caught black rats died over four to 17 days after consuming an average of 21.2 grams of bait (0.106 g of diphacinone) per animal. These trials indicated that a minimum average exposure time of 7 days with 37.5 g of bait is needed for effective control of black rats, and 6 days and 30 g are needed for effective control of Polynesian rats (Swift 1998).
- A broadcast application rate study using a nontoxic formulation of Ramik® Green and a biomarker determined the optimal application rate, 22.5 kg/ha or 20 lb/ac, which exposed 100% of Polynesian rats and 94.4% of black rats over a 14-day period (Dunlevy et al. 2000), even though immigration could not be eliminated. Bait disappearance was most rapid at the 22.5 kg/ha application rate with 50% of the bait disappearing by day 6 and 80% disappearing by day 12.
- An exposure study using remote cameras found that 98.98% of vertebrates photographed at broadcast rodenticide pellets were the target species, rats and mice (Dunlevy and Campbell 2002).
- A broadcast trial, also using Ramik® Green bait containing 0.005% (50 ppm) diphacinone, resulted in 100% control of radio-collared Polynesian, black, and brown rats in two 4-ha study areas in Hawai‘i (Lindsey and Forbes 2000). Follow-up broadcasts in the same study areas were also highly effective in controlling subsequent rat immigration.
- A trial of Ramik® Green broadcast into a 45.5 ha forested area in Hawai‘i also achieved 100% mortality of 21 radio-collared rats within one week of application. Three weeks after bait application, based on trapping and chew blocks, rat abundance was still reduced by 99% relative to reference areas (Spurr et al. 2003a and 2003b) despite the immigration issues of this main island study site.
- In the Bay of Islands, Adak, Alaska, a three-year study evaluated Ramik® Green and various application methods on several small islands (Dunlevy and Scharf 2008).

These successful laboratory trials and field studies strongly suggest that well planned rat eradication projects utilizing diphacinone have a very high probability of eradicating rats on islands if used appropriately.

Rodenticide Hazard Analysis

The US Environmental Protection Agency (EPA) evaluates the hazards associated with the use of rodenticides. Standard evaluation tests of hazard include a toxicity assessment of rodenticides from a single ingestion (acute toxicity) as well as with repeat ingestion over time (chronic toxicity), mortality of nontarget species, retention time of rodenticide residues in primary consumers (animals that eat the bait directly) and indirect exposure of predators and scavengers that eat exposed primary consumers. Because of these concerns, EPA requires standardized studies for determining the toxicity of compounds and their impacts on fish, birds and mammals prior to registration of a particular rodenticide formulation under FIFRA. EPA has two recent documents outlining study methodologies, overall results of studies, and resultant hazards of various rodenticides, including brodifacoum and diphacinone (Reregistration Eligibility Decision (US Environmental Protection Agency 1998) and Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach (Erickson and Urban 2004)). The following summary of study approaches and terms is primarily from Erickson and Urban (2004), which summarizes the findings of studies regarding diphacinone and brodifacoum, as well as other rodenticides.

The EPA limits their definition of nontarget hazard to a product of toxicity and exposure. The level of exposure is determined by the amount of active ingredient (a.i.) ingested.

Hazard can be characterized and assessed by many measures, including:

- Acute oral toxicity or LD_{50}– A single dose that is lethal to 50% of the test subjects in the population or study group under consideration, expressed as milligram(s) of active ingredient per kilogram of test subject body weight;
- Dietary toxicity or LC_{50}– The concentration of rodenticide in the diet (multiple feedings) that is lethal to 50% of test subjects in the population or study group under consideration, expressed as parts per million of the daily diet.
- Lowest observed effects level or LOEL– The lowest dosage at which measurable effects, such as increased blood-clotting times, are documented. This is not a mortality threshold and no negative impacts are necessarily derived at this hazard level. Diphacinone has LOELs calculated; brodifacoum does not because of its substantially higher toxicity.
- The dietary risk quotient (RQ) was developed by the EPA to compare hazards among different rodenticides. The ratio of the concentration of any rodenticide (ppm of active ingredient) to the dietary toxicity (LC_{50}) of the rodenticide provides a relative index of hazard. This allows for the comparison of the hazards among various rodenticides. The Level of Concern (LOC) is an RQ threshold used by the EPA to determine if unacceptable risk exists for a particular species. The index allows for comparisons among risks for different species. Risk is presumed for non-endangered species if the RQ is $\geq$0.5 and for an endangered species if the RQ >0.1.
- Half life - The length of time that rodenticide residues persist in tissues is calculated in terms of the time that half the original concentration of residue still persists in tissue or blood.

- Total daily food intake for a particular species compared to the animals weight can be used to gauge the possibility that an animal is physically capable of eating the amount of rodenticide (at any particular concentration of the active ingredient) required to deliver an LD_{50} dosage.

To describe the range of potential hazard to nontarget species from rodenticide application, this analysis discusses the acute oral toxicity of both diphacinone and brodifacoum for the species of concern. From the LD_{50} we can determine the amounts of bait and/or rodenticide residue in tissues of prey that an individual of a nontarget species would be required to eat to obtain this dosage. Using this information we can assess the potential for this level of exposure based on knowledge of the biology of the nontarget species, such as behavior and daily food intake. Another very useful way of evaluating the potential hazards associated with rodenticide use is to describe the lowest dosage which results in any measurable effect and assess the potential for this level of exposure. Using laboratory and field data accepted by the EPA, quantitative characterizations of rodenticide nontarget hazards can be made and assessed in conjunction with the known biology of the species of concern.

Standardized laboratory studies are used to determine the acute oral and dietary toxicity of vertebrate pesticides for some standard test subjects, such as brown rats, and sometimes for other species. These studies produce a range of values, sometimes with considerable variation. The details and assessments by the US EPA of these studies are discussed in the Reregistration Eligibility Decision (US EPA 1998) and Erickson and Urban (2004).

The determinations of the EPA in these documents are utilized in the analyses presented here. For untested mammals, a theoretical LD_{50} can be calculated, based on the weight of the animal, using the laboratory documented LD_{50}, accepted by the US EPA, for a brown rat for any particular compound. For a brown rat, the LD_{50} of diphacinone is 2.3 mg/kg; for brodifacoum it is 0.4 mg/kg, indicating the substantially greater relative toxicity for brodifacoum. A 100 kg mammal would, therefore, require 230 mg of diphacinone, or 40 mg of brodifacoum to ingest the projected LD_{50} dosage.

EPA calculates hazards for nontarget bird species the same way, using a known laboratory-derived LD_{50} from representative birds: the northern bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*). Some studies have also documented, in the laboratory, LD_{50} and LC_{50} values for some other species besides the standard species consistently used by EPA in toxicity studies.

Methodology Used in This Document to Analyze Rodenticide Impacts to Birds

The analyses of the direct and indirect impacts of diphacinone and brodifacoum on nontarget birds are based on the known laboratory LD_{50} and LC_{50} information documented by the US Environmental Protection Agency (US EPA 1998, Erickson and Urban 2004).

Broadcast applications of diphacinone bait at the maximum rate of 22.5 kg/ha (20 lb/ac); result in approximately one 2.25-gram pellet distributed about every square meter. The maximum proposed broadcast rate of brodifacoum bait is 18 kg/ha (16 pounds bait/acre),

resulting in a density of approximately one 2-gram pellet per square meter (see Section 2.1.3 for label requirements).

The analyses of the primary hazards of brodifacoum and diphacinone use a computed LD_{50}-equivalent dose. This is based on laboratory studies in species such as the rat, a surrogate for other mammals, and bobwhite or mallard for other avian species. The average weight of an adult female animal of concern and the established LD_{50} of the surrogate species studied are used to calculate the amount of each rodenticide that would need to be ingested to reach the LD_{50}-equivalent dosage. This is compared to the area over which that amount would be distributed during an aerial application and the likelihood of an animal eating every bait pellet within that area. If it is highly unlikely that the animal would directly eat bait pellets based on its dietary habits, the calculated results are evaluated in that context.

The analyses of the secondary impacts of brodifacoum and diphacinone assume that the adult female animal of average weight feeds exclusively in an area massively contaminated to the extent documented at the spill site in New Zealand and exclusively on the most contaminated samples collected during the monitoring of the incident: mussels and fish liver. One day after the accident, mussels contained brodifacoum residues of 0.41 ppm and a butterfish sampled nine days after the spill had brodifacoum liver residues of 0.04 ppm. This is then used to calculate the amounts of these prey items secondary nontarget species would need to eat in order to ingest the computed LD_{50} for the species of concern. This is then compared to either the animal's average daily food intake or body weight to determine if eating such a quantity is probable or even possible.

For the most conservative assessment of secondary hazard, it is assumed that nontarget species of concern would be exposed to prey items that have themselves been exposed to rodenticides and contain residues and that these residues are similar to the maximum residue levels of either potential prey items documented in Primus et al. (2005) during a massive point-source spill of rodenticide, laboratory exposure to a toxicant only, and/or collected from the site of an actual rodenticide operation.

The evaluation and comparison of LD_{50} values and risk quotients provides a good description of the upper end of the hazard spectrum associated with rodenticide use. However, because anticoagulants are far more toxic when administered on multiple days with smaller exposures, to fully characterize the range of possible hazard the lower end of the hazard potential needs to be assessed. To do this we will examine the Lowest Observed Effect Level (LOEL) for all nontarget species that we know are at the highest risk of exposure. Assessing the LOEL will illustrate the minimum amount of exposure necessary to produce a measurable effect, such as increased blood-clotting time. This is not a mortality threshold and no negative impacts are necessarily derived at this hazard level.

In a laboratory study using golden eagles fed diphacinone-laced sheep muscle (2.7 ppm) Savarie et al. (1979) established the LOEL for golden eagles at 0.11 mg/kg/day in a 7-day exposure study. The EPA reports the LOEL of diphacinone for rats in a 14-day subchronic lab study as 0.085 mg/kg/day (EPA 1998).

The LOELs of brodifacoum are not as well documented as those of diphacinone. No LOEL of brodifacoum for birds has been established because effects have been observed

for all doses administered in all tests. The EPA reports the LOEL of brodifacoum for rabbits in a developmental lab study as 0.005 mg/kg/day (EPA 1998). Using these available figures to extrapolate the LOELs for each of the species of concern the lower limit of potential hazard can be assessed.

Effects on Birds from Ingestion of Rodenticides by Eating Bait (Direct Effect)

Standard EPA studies of the acute oral toxicity of diphacinone and brodifacoum have been conducted for two avian species. For diphacinone, the LD_{50} for the mallard duck is 3,158 mg/kg and for the northern bobwhite 400 mg/kg $<LD_{50}<$ 2000 mg/kg. For brodifacoum, the LD_{50} for the mallard is 0.26 mg/kg (no documentation for the bobwhite) (Erickson and Urban 2004). The dietary (chronic) toxicity studies of diphacinone for mallard (*Anas* platyrhynchos) and bobwhite quail (*Colinus virginianus*) documented LC_{50} values of 906 ppm for the mallard and >5,000 ppm for the bobwhite quail. For brodifacoum, the LC_{50} reported for the mallard is 2.0 ppm and for the northern bobwhite it is 0.8 ppm, many orders of magnitude higher than the LC_{50} for diphacinone (Erickson and Urban 2004).

Primary and secondary hazard calculations of diphacinone acute oral toxicity for nongame birds weighing $\leq$0.22 pounds ($\leq$3.5 ounces) were made for the equivalent of Hawaiian passerine birds. In order to consume sufficient diphacinone bait to reach a dose equivalent to the LD_{50} for the northern bobwhite, a passerine bird would have to eat 0.53 pounds of bait or 5,027 pounds of invertebrates in one day. Neither of these amounts is even physically possible. While to obtain the LC_{50} for diphacinone, the bird would have to consume 0.36 g of bait or 3.59 g of invertebrates per day over several days. However, hazard calculations for sublethal exposure show that a 30 g bird would only need to eat 0.07 g (a 100^{th} of a bait pellet, or 0.2% of its body weight) or 0.65 g of invertebrates per day for multiple days to ingest a dose that resulted in measurable blood clotting effects in golden eagles. Therefore, small passerine birds could be vulnerable to sublethal or possibly lethal effects through both primary and secondary exposure if they forage on diphacinone bait or contaminated invertebrates over time (Eisemann and Swift 2006).

Birds that are most at risk from feeding directly on rodenticides are those that are naturally inquisitive, which are terrestrial ground-feeders, and that have a diet that includes grains and seeds. The risk of secondary poisoning is greatest for predatory and scavenging birds, especially those that feed directly on the target rodent species, such as owls. Brodifacoum has a far greater potential for primary and secondary poisoning of nontarget bird species than diphacinone because of its much higher toxicity, longer retention time in tissues, and higher rate of bioaccumulation (Erickson and Urban 2004, Eason and Wickstrom 2001, Fisher et al. 2003, Fisher et al. 2004). Combined with an extremely long half-life of residues in tissues, the general characteristic of anticoagulants for delayed symptoms and mortality after exposure results in target animals ingesting many lethal doses before death (Erickson and Urban 2004).

Erickson and Urban (2004) provide this useful discussion of potential effects of brodifacoum and diphacinone on avian nontarget species found during field operations:

> Eason and Spurr (1995) reviewed the impacts of brodifacoum baiting on nontarget birds during baiting programs in New Zealand, where bait is

applied in bait stations (50 ppm cereal-based wax blocks) or aerially broadcast (20 ppm pellets). They report mortality of a wide range of bird species, including 33 indigenous species or subspecies and 8 introduced species or subspecies, and presume most resulted from primary exposure. Populations of indigenous rails (weka, *Gallirallus australus*; pukeko, *Porphyrio porphyrio*) monitored during rodenticide baiting operations were severely reduced: "For example, the entire population of western weka on Tawhitinui island were exterminated by consumption of Talon® 50WB intended for ship rats [a brodifacoum formulation], which they obtained by reaching into bait stations, eating bait dropped by rats, and eating dead or dying rats (Taylor 1984)."

On another island, 80% to 90% of the Stewart Island weka population was killed by brodifacoum bait applied for brown rats. Aerial application of 0.002% brodifacoum bait on two other islands reduced a weka population by about 98% and a pukeko population by >90%. Numbers of quail, blackbirds, sparrows and myna were markedly reduced on another island. Some other species suffered no apparent adverse effects. Dowding et al. (1999) and Veitch (2002) found numerous dead birds after an aerial baiting operation to eradicate rats and mice and reduce rabbit numbers on Motuihe Island, New Zealand. Brodifacoum bait (20ppm) was applied twice, with 9 days between applications. Nontarget species were monitored, including pukeka (3 groups of 98 birds), a flock of 52 paradise shelducks (*Tadorna variegata*), 8 New Zealand dotterels (*Charadrius obscurus*), and 14 variable oystercatchers (*Haematopus unicolor*). There was no evidence that dotterels or oystercatchers were adversely affected, but mortality of pukeko and shelducks was 49% and 60%, respectively. Birds of 10 species were found dead. The liver from each of 29 dead birds of 10 species was analyzed. All livers contained brodifacoum residue, with mean levels per species ranging from 0.56 to 1.43 ppm. Chaffinch (*Fringilla coelebs*), North Island robin (*Petroica australis longipes*), North Island weka, and North Island saddleback (*Philesturnus carunculatus rufusater*) also were found dead after a brodifacoum baiting on Mokoia Island, New Zealand (Stephenson et al.1999).

Hegdal (1985) conducted a field study in Washington to examine the risk to game birds from the broadcast application of 0.005% diphacinone bait applied for vole control in orchards. Most orchards were treated twice, with 20 to 30 days between treatments; at an average rate of 12.9 kg/ha (11.5 lb/acre). Telemetry was used to monitor the fate of 52 ring-necked pheasants, 18 California quail, and 30 chukar potentially exposed to the bait. About half of the quail and all chukar were pen-raised and had been released into the orchards. Dead game birds and other animals found were necropsied and any available tissue collected for residue analysis. Eight of 30 pheasants, 9 of 15 quail and one of ten chukar collected by the researchers or shot by hunters contained diphacinone residue in the liver but no mortalities were attributed to diphacinone. Bait made up as much as 90% of crop contents of some birds. No residue was detected in four

passerines collected 31 to 73 days after treatment. The author concluded that risk to game birds in orchards appeared to be low but emphasized that substantial quantities of bait were eaten and longer-term behavioral and physiological effects, such as susceptibility to predation, need to be considered along with direct mortality in order to evaluate potential hazards from exposure.

Several laboratory studies document data assessing the hazards of rodenticides ingested by birds. Chickens (*Gallus gallus*) were fed a rodenticide containing 50 ppm brodifacoum by Lund (1981). This study was a choice test and included offering of the toxic bait as well as untreated chicken food for up to 15 days. The four chickens offered brodifacoum bait died within 6 to 12 days. A similar study with chickens by Christopher et al. (1984) offered brodifacoum bait every other day for one to four feedings and documented 50% mortality. Ten northern bobwhites and 10 ring-necked pheasants were exposed to a 50 ppm brodifacoum rodenticide for 14 days in an *ad libitum* feeding choice including the toxic pellets and untreated food by Ross et al. (1979a) and Ross et al. (1979(b)). Six each of the bobwhites and pheasants died. In addition, several pheasants died when exposed to 50 ppm brodifacoum pellets in a broadcast pen trial conducted by ICI Americas, Inc (1981). Diphacinone was not tested in any of these studies.

During field studies using diphacinone, searches for nontarget carcasses after baiting found one dove and two roadrunners (*Geococcyx californicus*); however there was no evidence that these birds were exposed to the rodenticide (Baroch 1994 and 1996). No avian nontarget mortality was observed during rodent eradication operations using a diphacinone rodenticide conducted on Buck Island in the Virgin Islands (Witmer et al. 2001) or Canna Island in Scotland (Elizabeth Bell, pers. comm., February 2006). Throughout two years of studies using a diphacinone rodenticide in the Aleutian Islands only one bird carcass was documented, though two ravens shot during this work also contained diphacinone residues and winter wrens, song sparrows and ptarmigan were also documented to eat the bait (Dunlevy and Scharf 2008). Two studies evaluated diphacinone residues in game birds captured from sites in Hawai‘i that had been treated by hand or aerial broadcasting 0.005% diphacinone bait. The first study utilized hand broadcast techniques on a 10-acre treatment area (Spurr et al. 2003a). Five Kalij pheasants (*Lophura leucomelana*) were collected within the treatment area between 2 and 6 weeks after treatment. Of the five, only one contained detectable diphacinone residues. The liver of this bird contained 0.09 ppm diphacinone. The second study was an aerial broadcast trial of Ramik Green (Spurr et al. 2003b). Two Kalij pheasants were collected within the 112 acre treatment area one month after treatment. Diphacinone residues of 0.12 and 0.18 ppm were found in the livers of these birds. Though extensive carcass searches were conducted during both studies no avian mortality due to diphacinone was found.

Effects on Birds from Rodenticide Ingestion by Eating Prey (Indirect Effect)

Incident reports submitted to EPA indicate that nontarget birds and mammals are being secondarily exposed to rodenticides, especially brodifacoum, in the field. Brodifacoum is widely used for control of rodents in protective stations around buildings and human habitation; while diphacinone products are also available for this use pattern they are

used less for this purpose. Diphacinone products are also registered for some field uses, such as in the agriculture industry. In 264 reported incidents, 20 animals had diphacinone residues and 244 animals had brodifacoum residues. The birds most commonly exposed to brodifacoum include great horned owls and red-tailed hawks, but multiple incidents are reported for bald and golden eagles, crows, barn owls, screech owls, hawks, falcons, kestrels and vultures.

Erickson and Urban (2004) found eleven laboratory studies which have investigated brodifacoum secondary hazards in eight nontarget avian species. These studies recorded that out of a total of 149 individuals that were exposed to brodifacoum-poisoned prey, 63 birds (42% of the total) died, including: eleven of twenty barn owls, six of six red-tailed hawks (*Buteo jamaicensis*) and red-shouldered hawks (*Buteo lineatus*), thirteen of 65 American kestrels (*Falco sparverius*), one of four Eurasian harriers (*Circus pygargus*), and 32 of 50 laughing gulls (*Larus atricilla*). However, no deaths occurred in four golden eagles tested (*Aquila chrysactos*), although three showed external symptoms of anticoagulant toxicosis such as bleeding. Some studies did not report whether evidence of toxicosis was observed in surviving birds. Of studies that examined survivors, about one-third exhibited symptoms of toxicosis. Stone et al. (1999) also found brodifacoum residues in wildlife carcasses submitted for testing in New York State.

Three laboratory studies report the secondary toxicity of diphacinone to birds. Test species were barn owls, great horned owls (*Bubo virginianus*), saw-whet owls (*Aegolius acadicus*), golden eagles (*Aquila chrysaetos*), and American crows (*Corvus brachyrhynchos*). A total of 34 individuals were exposed to diphacinone-poisoned prey during these studies and three (9%) birds died, including two of three great horned owls and the only saw-whet owl tested. Symptoms of anticoagulant poisoning were noted in 13 (42%) of the survivors, indicating that raptors can recover from sublethal doses. The highest dosage administered to an eagle was 0.23 mg/kg/day for 10 consecutive days and the LOEL was determined to be 0.11 mg/kg/day. If it is assumed that the great horned owls ate equal quantities of treated mice each day, they would have consumed a maximum dose of 0.78 mg/kg/day for 5 days. Using the same methods, it can be calculated that the saw-whet owl consumed a dose of 11.1 mg/kg/day (Erickson and Urban 2004).

Hazard calculations for the short-eared owl (*Asio flammeus,* pueo) from eating contaminated rats were calculated for the secondary effects of diphacinone as there is an extremely low probability that an owl would feed directly on bait pellets. A 0.77 pound bird would have to consume at least 90.5 pounds of rodents containing 3.4 ppm diphacinone (the highest whole-carcass residue found in a rat) in one day to ingest a dose equivalent to the LD_{50} for the northern bobwhite. Hazard calculations for sublethal exposure show that an owl would only need to eat 11 g of rodent tissue containing 3.4 ppm diphacinone per day for multiple days to ingest a LOEL dose. This amount is less than one rodent per day (Eisemann and Swift 2006). The assessments in Eisemann and Swift (2006) are based on very conservative assumptions and are assumed to overestimate the actual hazard of aerial broadcast of diphacinone.

Conclusion on Rodenticide Toxicity to Birds

The EPA (1998) states that brodifacoum is "very highly toxic" to both bobwhite quail and mallard duck for both acute and dietary exposure. Diphacinone is "moderately toxic" in acute tests of bobwhite quail, "practically nontoxic" to quail in dietary tests, and "moderately toxic" to mallard in dietary tests. Brodifacoum toxicity in birds is two orders of magnitude more toxic than required for the category "very highly toxic." The EPA declares a potential primary hazard to nontarget birds when their dietary risk quotient equals or exceeds 0.5 for non-endangered species and 0.1 for endangered species. Brodifacoum exceeds this level of concern for non-endangered species by 126-fold using the northern bobwhite LC_{50} and 50-fold using the mallard LC_{50}. For endangered species, the level of concern is exceeded by 630 times and 250 times, respectively. Diphacinone does not exceed these levels of concern for either endangered or non-endangered species using the mallard LC_{50}. Using the northern bobwhite $LC_{50,}$ diphacinone is considered "practically nontoxic" to birds by the EPA. The LOEL of brodifacoum for birds has not been determined; where efforts to establish this have been made, all dosages administered produced measurable effects; therefore a dosage where no observed effects (NOEL) have been measured has not been documented. A dosage of no observed effects is necessary to establish the lowest observable effects level.

Although individuals of avian nontarget species can die during eradication operations, especially associated with the use of brodifacoum, if the nontarget population is not extirpated and is healthy and viable it usually recovers. However, if the population is an endangered species or a small isolated island population, it may be driven too low to recover or experience negative population-level genetic effects. In most cases the long-term ecosystem benefits probably outweigh the initial nontarget mortality caused by rodenticides during eradication operations (Taylor and Thomas 1993, Eason and Spurr 1995, Dowding et al. 1999). Stephenson et al. (1999) found that passerine populations can recover naturally from a 30% decrease in populations within one to two breeding seasons following a rodenticide operation because passerine species typically have several clutches per year and successfully fledge several young per clutch. Populations of owls, because they live longer and typically fledge less than one chick per year, may recover more slowly, taking two to three seasons (also Murphy et al. 1998). The relative resilience of a species to recover after large population declines depends on the species capacity to compensate for density independent perturbations in abundance, such as the broad-scale application of rodenticides. Species with a high intrinsic rate of increase and strong-density dependent links between their demographics and factors that regulate their abundance will typically be more resilient than species without these population dynamics. Species for which there is clear evidence of a high intrinsic capacity for increase and strong density-dependence in their dynamics should be able to sustain higher levels of reduction from poisoning without any undue threat to their long-term viability (Choquenot and Ruscoe 1999).

Erickson and Urban (2004) conclude that potential primary risks are higher for second generation rodenticides, including brodifacoum, than for first generation rodenticides, including diphacinone. A small bird finding and eating just a small pellet or two of brodifacoum is likely to ingest a lethal dose, and a few small pellets could provide a lethal dose to larger birds. In contrast, it seems highly unlikely that any small bird could

eat 100 to 1000 pellets of diphacinone in a single feeding which would be needed to provide an LD_{50} dose from a first-generation anticoagulant. Eason et al. (1999) and Eason and Wickstrom (2001) state: "the recorded mortality of birds after some control operations, coupled with the detection of brodifacoum residues in a range of wildlife including native birds and feral game animals raises serious concerns about the long-term effects of the targeted field use of brodifacoum…where wildlife might encounter poisoned carcasses." New Zealand is recommending reducing the field use of brodifacoum because of the high risk of poisoning nontarget species, especially secondary poisoning (Eason and Wickstrom 2001, Eason and Murphy 2001, Hoare and Hare 2006).

APPENDIX B

APPROVED PESTICIDE LABELS FOR DIPHACINONE AND BRODIFACOUM

RESTRICTED USE PESTICIDE
DUE TO HAZARDS TO NON-TARGET SPECIES

For retail sale to and use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicators certification

For use by or in cooperation with government conservation agencies.

Department of Agriculture
STATE OF HAWAII

LICENSED

PERIOD 2008-2010 LIC. NO. 8600.1

Diphacinone-50:
Pelleted Rodenticide Bait for Conservation Purposes

Fish Flavored, Weather-resistant Rodenticide for Control or Eradication of Invasive Rodents on Islands or Vessels for Conservation Purposes

ACTIVE INGREDIENT:	
Diphacinone (2-Diphenylacetyl-1,3-Indandione)	0.005%
INERT INGREDIENTS:	99.995%
TOTAL	100.000%

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS

HAZARD TO HUMANS AND DOMESTIC ANIMALS

Caution: Keep away from humans, domestic animals and pets. If swallowed, this material may reduce the clotting ability of the blood and cause bleeding. Wear protective gloves when applying or loading bait. With a detergent and hot water, wash all implements used for applying bait. Do not use these implements for mixing, holding or transferring food or feed.

FIRST AID Have label with you when obtaining treatment advice.	
If swallowed	• Call a poison control center, doctor, or 1-800-222-1222 immediately for treatment advice. • Have person sip a glass of water if able to swallow. • Do not induce vomiting unless told to do so by the poison control center or doctor.
If on skin or clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center, doctor, or 1-800-222-1222 immediately for treatment advice.
• **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.	

For a medical emergency involving this product, call 1-800-222-1222.

ENVIRONMENTAL HAZARDS

This product is toxic to mammals and birds. Predatory and scavenging mammals and birds might be poisoned if they feed upon animals that have eaten bait.

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.

DIRECTIONS FOR USE

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

READ THIS LABEL: Read this entire label and follow all use directions and use precautions.

IMPORTANT: Do not expose children or pets to this product. Take all appropriate steps to limit exposure to and impacts on nontarget species, especially those for which special conservation efforts are planned or ongoing. To help to prevent accidents:

1) Store product not in use in a location out of reach of children and pets.
2) Apply bait only as specified on this label and in strict accordance with the "**USE RESTRICTIONS:**" and "**APPLICATION DIRECTIONS:**". For applications involving bait stations, the bait stations must be tamper-resistant. The bait stations must deny access to bait compartments by children, pets, and other non-target species larger in body size than the type(s) of rats or mice being targeted by the bait program. Lock and secure bait stations, as necessary, to exclude such nontarget species. In locations where captive or feral livestock occur, either remove and exclude such animals from the application site prior to treatment or make sure that the bait stations used are capable of denying them access to bait compartments, and
3) Dispose of product container, and unused, spoiled and unconsumed bait as specified on this label.

USE RESTRICTIONS: This product may be used only to control or eradicate Norway rats (*Rattus norvegicus)*, roof rats (*Rattus rattus),* Polynesian rats (*Rattus exulans),* house mice (*Mus musculus*) or other types of invasive rodents for conservation purposes on islands, grounded vessels or vessels in peril of grounding. This product may be applied only using bait stations, burrow baiting, canopy baiting or aerial and ground broadcast application techniques.

This product is to be used for the protection of State or Federally-listed Threatened or Endangered Species or other species determined to require special protection.

Do not apply this product to food or feed.

Treated areas must be posted with warning signs appropriate to the current rodent control project.

APPLICATION DIRECTIONS:

Bait Stations: Tamper-resistant bait stations must be used when applying this product on grounded vessels or vessels in peril of grounding or when used in areas of human habitation. See Item 2) under "**IMPORTANT:**" regarding the performance characteristics needed for tamper-resistant bait stations. To bait rats: Apply 4 to 16 ounces (113 to 454 grams) of bait per placement. Space placements at intervals of 5 to 50 meters. Placements should be made in a grid over the area for which rodent control is desired. To bait mice: Apply 0.25 to 0.5 ounces (7 to 14 grams) of bait per placement. Space placements at intervals of 2 to 4 meters. Placements should be made in a grid over the area for which rodent control is desired. Larger placements (up to 2 ounces) may be needed at points of very high mouse activity. For both rat and mouse baiting: Maintain an uninterrupted supply of fresh bait for at least 15 days or until signs of rodent activity cease. Where a continuous source of infestation is present, permanent bait stations may be established and bait replenished as needed.

Burrow-baiting: Place bait in burrows only if this can be done in a way that minimizes potential for ejection of bait and exposure of bait to seed-eating birds and other non-target species. To bait rats: place 3 to 4 ounces (85 to 113 g) of bait inside each burrow entrance. Baits used in burrows may be applied in piles or in cloth or reasealable plastic bags. The bags should be knotted or otherwise sealed to avoid spillage and holes should be made in plastic bags to allow the bait odor to escape. To bait mice: place approximately 0.25 ounces (7 grams) of bait in each active burrow. For both rat and mouse baiting: place one such bag or placement in each active burrow opening and push bag into burrow far enough so that its presence can barely be seen. Do not plug burrows. Flag treated burrows and inspect them frequently, daily if possible. Maintain an uninterrupted supply of bait for at least 15 days or until rodent activity ceases. Remove bait from burrows if there is evidence that bags are ejected.

Canopy Baiting (bait placement in the canopy of trees and shrubs): In areas where sufficient food and cover are available to harbor populations of rodents in canopies of trees and shrubs, canopy baiting should be included in the baiting strategy. Approximately 4 to 7 ounces (113 g to 200 g) of bait should be placed in a cloth or resealable plastic bag. The bags should be knotted or otherwise sealed to avoid spillage and holes should be made in plastic bags to allow the bait odor to escape. Using long poles (or other devices) or by hand, bait filled bags should be placed in the canopy of trees or shrubs. Baits should be placed in the canopy at intervals of 50 meters or less, depending upon the level of rodent infestation in these habitats. In

some vegetation types, bait stations may need to be used to ensure bait will stay in the canopy.

Aerial and Ground Broadcast: Broadcast applications are prohibited on vessels or in areas of human habitation. Broadcast bait pellets by helicopter or manually at a rate of 10 to 12.5 lbs. of bait per acre (11.1 to 13.8 kg/ha) per treatment. Make a second broadcast application typically 5 to 7 days after the first application, depending upon local weather conditions, at a rate no higher than 12.5 lbs. (13.8 g/ha) of bait per acre. In situations where weather or logistics only allow one bait application, a single application may be made at a rate no higher than 20.0 lbs. bait per acre (22.5 kg/ha).

Aerial (helicopter) applications may not be made in winds higher than 35 mph (30 knots). Pilot in command has final authority for determining safe flying conditions. However, aerial applications will be terminated when the following conditions are met:

- Windspeed in excess of 25 knots with an evaluation of the terrain and impact of the wind conditions and not to exceed a steady wind velocity of 30 knots.

If rat activity persists after broadcast application, set up and maintain tamper-resistant bait stations or apply bait directly to rodent burrows in areas where rodents remain active. If terrain does not permit use of bait station or burrow baiting, continue with broadcast baiting, limiting such treatments to areas where active signs of rats are seen. Maintain treatments for as long as rodent activity is evident in the area and rodents appear to be accepting bait.

For all methods of baiting, monitor the baited area periodically and, using gloves, collect and dispose of any dead animals and spilled bait properly. Dead animals and spilled bait may be buried on site if the depth of burial makes excavation by nontarget animals extremely unlikely.

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
4700 River Road, Unit 149
Riverdale, MD 20737-1237
EPA Reg. No 56228-35
EPA Est. No. 61282-WI-1

Net Contents: 20 lbs. (9.07 Kg)

Label Revised: 12/07/2007

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

Keep away from humans, domestic animals and pets. If swallowed, this material may reduce the clotting ability of the blood and cause bleeding. Wear protective gloves when applying or loading bait. With detergent and hot water, wash all implements used for applying bait. Do not use these implements for mixing, holding, or transferring food or feed.

ENVIRONMENTAL HAZARDS

This pesticide is toxic to birds, mammals and aquatic organisms. Predatory and scavenging mammals and birds might be poisoned if they feed upon animals that have eaten bait.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear:

-long sleeved shirt and long pants
-gloves
-shoes plus socks

For aerial application, in addition to the above PPE, loaders must wear protective eyewear or a face shield and a dust/mist filtering respirator (MSHA/NIOSH TC-21C).

USE RESTRICTIONS

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time that the product is applied.

READ THIS LABEL: Read this entire label and follow all use directions and precautions**.**

IMPORTANT: Do not expose children, pets or other non-target animals to rodenticides. To help prevent accidents:

1) Keep children out of areas where this product is used or deny them access to bait by use of tamper resistant bait stations.
2) Store this product in locations out of reach of children, pets, and other nontarget animals.
3) Apply bait only according to the directions authorized.
4) Dispose of product container and unused, spoiled, or unconsumed bait as specified in the "**STORAGE AND DISPOSAL**" section.

(SEE RIGHT PANEL FOR ADDITIONAL USE RESTRICTIONS)

RESTRICTED USE PESTICIDE

DUE TO HAZARDS TO NON-TARGET SPECIES

For retail sale to and use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicators certification.

For use by or in cooperation with government conservation agencies.

BRODIFACOUM-25D CONSERVATION

PELLETED RODENTICIDE BAIT FOR CONSERVATION PURPOSES

For control or eradication of invasive rodents in dry climates on islands or vessels for conservation purposes

ACTIVE INGREDIENT
Brodifacoum (CAS No. 56073-10-0) 0.0025%
INERT INGREDIENTS 99.9975%
TOTAL 100.0000%

KEEP OUT OF REACH OF CHILDREN

CAUTION

First Aid

If swallowed	-Call a physician or poison control center immediately for treatment advice. -Have person sip a glass of water if able to swallow. -Do not induce vomiting unless told to do so by a poison control center or doctor. -Do not give anything by mouth to an unconscious person.
If on skin or clothing	-Take off contaminated clothing. -Rinse skin immediately with plenty of water for 15-20 minutes. -Call a poison control center or doctor for treatment advice.
If inhaled	-Move person to fresh air. -If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. -Call a poison control center or doctor for further treatment advice.
If in eyes	-Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. -Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor, or when going for treatment.

For a medical emergency involving this product, call (877) 854-2494

NOTE TO PHYSICIAN: If swallowed, this material may reduce the clotting ability of blood and cause bleeding. If ingested, administer Vitamin K_1, intramuscularly or orally, as indicated in bishydroxycoumarin overdose. Repeat as necessary based on monitoring of prothrombin times.

USE RESTRICTIONS, (CONT)

This product may be used to control or eradicate Norway rats (*Rattus norvegicus*), roof rats (*Rattus rattus*), Polynesian rats (*Rattus exulans*), house mice (*Mus musculus*) or other types of invasive rodents on islands for conservation purposes, or on grounded vessels or vessels in peril of grounding.

This product may be applied using bait stations, burrow baiting, canopy baiting or by aerial and ground broadcast application techniques.

This product is to be used for the protection of State or Federally-listed Threatened or Endangered Species or other species determined to require special protection.

Do not apply this product to food or feed.

Treated areas must be posted with warning signs appropriate to the current rodent control project.

This product is for use in dry climates.

DIRECTIONS FOR USE

BAIT STATIONS: Tamper-resistant bait stations must be used when applying this product to grounded vessels or vessels in peril of grounding, or when used in areas of human habitation. Bait must be applied in locations out of reach of children, non-target wildlife, or domestic animals, or in tamper-resistant bait stations.

TO BAIT RATS: Apply 4 to 16 ounces (113 to 454 grams) of bait per placement. Space placements at intervals of 16 to 160 ft (about 5 to 50 meters). Placements should be made in a grid over the area for which rodent control is desired.

TO BAIT MICE: Apply 0.25 to 0.5 ounces (7 to 14 grams) of bait per placement. Space placements at intervals of 6 to 12 ft (about 2 to 4 meters). Larger placements, up to 2 ounces (57 grams) may be needed at points of very high mouse activity. Placements should be made in a grid over the area for which rodent control is desired.

FOR BOTH RAT AND MOUSE BAITING: Maintain an uninterrupted supply of fresh bait for at least 15 days or until signs of rodent activity cease. Where a continuous source of infestation is present, permanent bait stations may be established and bait replenished as needed.

EPA Approved 03/18/08
EPA Reg. No. 56228-37

DIRECTIONS FOR USE (CONT.)

BURROW-BAITING: Place bait in burrows only if this can be done in a way that minimizes potential for ejection of bait and exposure of bait non-target species.
TO BAIT RATS: Place 3 to 4 ounces (85 to 113 g) of bait inside each burrow entrance. Baits used in burrows may be applied in piles or in cloth or resealable plastic bags. The bags should be knotted or otherwise sealed to avoid spillage and holes should be made in plastic bags to allow the bait odor to escape.
TO BAIT MICE: Place approximately 0.25 ounces (7 grams) of bait in a cloth or resealable bag in each active burrow.
FOR BOTH RAT AND MOUSE BAITING: Place one such bag or placement in each active burrow opening and push bag into burrow far enough so that its presence can barely be seen. Do not plug burrows. Flag treated burrows and inspect them frequently, daily if possible. Maintain an uninterrupted supply of bait for at least 15 days or until rodent activity ceases. Remove bait from burrows if there is evidence that bags are ejected.

CANOPY BAITING (bait placement in the canopy of trees and shrubs): In areas where sufficient food and cover are available to harbor populations of rodents in canopies of trees and shrubs, canopy baiting should be included in the baiting strategy. Approximately 4 to 7 ounces (113 to 200 grams) of bait should be placed in a cloth or resealable plastic bag. The bags should be knotted or otherwise sealed to avoid spillage and holes should be made in plastic bags to allow the bait odor to escape. Using long poles (or other devices) or by hand, bait filled bags should be placed in the canopy of trees or shrubs. Baits should be placed in the canopy at intervals of 160 ft (about 50 meters) or less, depending upon the level of rodent infestation in these habitats. In some vegetation types, bait stations may need to be used to ensure bait will stay in the canopy.

DIRECTIONS FOR USE (CONT.)

BROADCAST APPLICATION: Broadcast applications are prohibited on vessels or in areas of human habitation. Broadcast bait using aircraft, ground-based mechanical equipment, or by gloved hand at a rate no greater than 16 lbs of bait per acre (18 kg bait/hectare) per application. Make a second broadcast application, typically 5 to 7 days after the first application, depending on local weather conditions, at a rate no higher than 8 lbs. of bait per acre (9 kg bait/hectare). In situations where weather or logistics only allow one bait application, a single application may be made at a rate no higher than 16 lbs. bait per acre (18 kg/ha).

Aerial (helicopter) applications may not be made in winds higher than 35 mph (30 knots). Pilot in command has final authority for determining safe flying conditions. However, aerial applications will be terminated when the following conditions are present:

> Windspeed in excess of 25 knots with an evaluation of the terrain and impact of the wind conditions and not to exceed a steady wind velocity of 30 knots.

Set the application rate according to the extent of the infestation and apparent population density. For eradication operations, treat entire land masses.

Assess baited areas for signs of residual rodent activity (typically 7 to 10 days post-treatment). If rodent activity persists, set up and maintain tamper-resistant bait stations or apply bait directly to rodent burrows in areas where rodents remain active. If terrain does not permit use of bait stations or burrow baiting, continue with broadcast baiting, limiting such treatments to areas where active signs of rodents are seen. Maintain treatments for as long as rodent activity is evident in the area and rodents appear to be accepting bait.

For all methods of baiting, monitor the baited area periodically and, using gloves, collect and dispose of any dead animals and spilled bait properly.

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 unauthorized people, 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 at an approved waste disposal facility.

CONTAINER DISPOSAL: Completely empty container. Then dispose of empty container in sanitary landfill or by incineration, or, if allowed by State and local authorities, by burning. If burned, stay out of smoke.

NOTICE: Buyer assumes all risks of use, storage, or handling of the material not in strict accordance with directions given herewith. The efficacy of the product may be reduced under high moisture conditions.

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Riverdale, MD 20737-1237
EPA Est. No. 56228-ID-1
EPA Reg. No. 56228-37
Net Weight

APPENDIX C

RESULTS OF LABORATORY ANALYSIS OF MARINE SAMPLES COLLECTED AFTER THE 2008 AERIAL DIPHACINONE APPLICATION TO MOKAPU ISLAND, MOLOKA‘I

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 08-025/1 Date: 04/03/08 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Peter Dunlevy
Pacific Islands Fish and Wildlife Office
USDA – APHIS – Wildlife Services

Katie Swift
Ecological Services Office
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Seawater

Method: 158A - Modified

Analysis Date: 03/27/08

AC Notebook Reference: AC 137 pp. 169-170

QC Notebook Reference: QC 26 pp. 66

Analyst: Chad Wermager, Tom Primus

Sample Description: Water samples arrived 03/20/2008 and were logged into our sample tracking system. Water samples were in 250 mL screw top jars. Water samples were stored in a refrigerator at 4 °C until analyzed. All samples were analyzed with a modified version of method 158A. The method uses 150 mL of sample. As specified 75 mL of each set of two replicates from each sample location (total of six) were composited into a 150 mL sample. The remaining water from each of 12 samples (two from each location) was composited after the final results were tabulated. This composited sample will be used for a storage stability study.

Additional Comments: The MLOD was 0.029 ppb Diphacinone and 0.058 ppb Chlorophacinone. Method 158A modifications included omitting step 3 (addition of salt to the sample to increase ionic strength of the sample) and replacing the mobile phase with 60% 5 mM TBA in Methanol : 40% Aqueous IPCA Solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QC Specialist	Date	Reviewer	Date
	4/19/08		4/19/08		4/19/08

Results:

Table 1. Diphacinone concentration in analyzed water samples.

Sample Description	Lab ID	Diphacinone Conc. (ppb)
Kalaupapa Reference Sea Water	S080320-01	<MLOD
Mokapu Sea Water 2/17 1A	S080320-02	<MLOD
Mokapu Sea Water 2/17 1B	S080320-03	
Mokapu Sea Water 2/17 2A	S080320-04	<MLOD
Mokapu Sea Water 2/17 2B	S080320-05	
Mokapu Sea Water 2/17 3A	S080320-06	<MLOD
Mokapu Sea Water 2/17 3B	S080320-07	
Mokapu Sea Water 2/17 4A	S080320-08	<MLOD
Mokapu Sea Water 2/17 4B	S080320-09	
Mokapu Sea Water 2/17 5A	S080320-10	<MLOD
Mokapu Sea Water 2/17 5B	S080320-11	
Mokapu Sea Water 2/17 6A	S080320-12	<MLOD
Mokapu Sea Water 2/17 6B	S080320-13	

75 mL of each sample designated as A and B were composited together for each 150 mL sample.

Table 2. Quality Control Recovery for Diphacinone (Surrogate Corrected).

ID	Fortification Level (ppb)	% Recovery (surrogate corrected)
QW 1	Blank	------
QW 2	Blank	------
QW 3	0.502	115
QW 4	0.500	114
QW 5	2.00	111
QW 6	2.00	103
Mean		111 ± 5.4

Kalaupapa Reference Sea Water used for all QC samples (S080320-01

Cc:
Tom Primus
Doreen Griffin

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 08-025/2 Date: 04/03/2008 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Peter Dunlevy
Pacific Islands Fish and Wildlife Office
USDA – APHIS – Wildlife Services

Katie Swift
Ecological Services Office
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Fish Tissue

Method: 159A - Modified

Analysis Date: 3/31/08

AC Notebook Reference: AC 137 pp. 171-173

QC Notebook Reference: QC 26 p. 67

Analyst: Chad Wermager, Tom Primus

Sample Description: Fish samples arrived 03/20/08 and were logged into our sample tracking system. Samples arrived in Ziploc bags according to sample number with fish fillet individually wrapped in aluminum foil. Each tissue sample was homogenized in a SPEX liquid nitrogen freezer mill. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-30 °C) until analyzed.

Additional Comments: The MLOD was determined to be 0.013 ppm Diphacinone and 0.003 ppm Chlorophacinone. Modifications to method 159A included the following. After evaporating the extraction solution, each sample residue was reconstituted with 2 mL chloroform and 3 mL hexanes. During filtering before cleanup, each sample tube was rinsed with 1 mL of both chloroform and hexanes. The solid phase extraction (SPE) cleanup procedure was completed with Phenomenex Strata X-AW 33 µm polymeric weak anion (200 mg) SPE columns conditioned with 0.5 mL methanol, 1.0 mL chloroform and 1.5 mL hexanes. After loading each SPE column with the sample extract, each column was washed with a solution used to rinse the sample tube consisting of 0.25 mL methanol, 0.5 mL chloroform and 0.75 mL hexanes. The analyte was eluted off each SPE column with 12 mL of 15 mM TBA in methanol and collected in a 10 mL screw top tube.

The mobile phase was replaced with 60% 5 mM TBA in Methanol : 40% Aqueous IPCA Solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	4/19/08 Date	QC Specialist	4/19/08 Date	Reviewer	4/19/08 Date

Results:

Table 1. Diphacinone concentration in analyzed fish samples.

Sample Description	Lab ID	Diphacinone Conc. (ppm)
Oahu Fish Market Reference Fish	S080320-14	<MLOD
Mokapu 2/17 Fish 1	S080320-15	<MLOD
Mokapu 2/17 Fish 2	S080320-16	<MLOD
Mokapu 2/17 Fish 3	S080320-17	<MLOD
Mokapu 2/17 Fish 4	S080320-18	<MLOD
Mokapu 2/17 Fish 5	S080320-19	<MLOD
Mokapu 2/17 Fish 6	S080320-20	<MLOD

Table 2. Quality Control Recovery for Diphacinone (Surrogate Corrected).

ID	Fortification Level (ppm)	% Recovery (surrogate corrected)
QF 1	Blank	------
QF 2	Blank	------
QF 3	0.100	97.5
QF 4	0.0947	100
QF 5	0.237	103
QF 6	0.244	100
Mean		100 ± 2.3

Oahu Fish Market Reference Fish used for all QC samples (S080320-14)

Cc: Tom Primus
Doreen Griffin
John Johnston

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 08-025/3 Date: 04/21/2008 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Peter Dunlevy
Pacific Islands Fish and Wildlife Office
USDA – APHIS – Wildlife Services

Katie Swift
Ecological Services Office
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Limpets

Method: 159A - Modified

Analysis Date: 4/14/08

AC Notebook Reference: AC 137 pp. 171, 175

QC Notebook Reference: QC 26 p. 71

Analyst: Chad Wermager, Tom Primus

Sample Description: Limpet samples arrived 03/20/08 and were logged into our sample tracking system. Samples arrived in Ziploc bags according to sample number with limpet soft tissue wrapped in aluminum foil. Samples had no shell. Reference limpets (S080320-21) required soft tissue to be removed from shell before homogenization. Each tissue sample was homogenized in a SPEX liquid nitrogen freezer mill. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-30 °C) until analyzed.

Additional Comments: The MLOD was determined to be 0.059 ppm Diphacinone. Modifications to method 159A included the following. Methanol was used as the extraction solution. After evaporating the extraction solution, each sample residue was reconstituted with 2 mL chloroform and 3 mL hexanes. During filtering before cleanup, each sample tube was rinsed with 1 mL of both chloroform and hexanes. The solid phase extraction (SPE) cleanup procedure was completed with Phenomenex Strata X-AW 33 µm polymeric weak anion (500 mg) SPE columns conditioned with 1.5 mL chloroform and 1.75 mL hexanes. After loading each SPE column with the sample extract, each column was washed with a solution used to rinse the sample tube consisting of 1.5 mL chloroform and 1.75 mL hexanes. The analyte was eluted off each SPE column with 12 mL of 15 mM TBA in methanol and collected in a 10 mL screw top tube.

The mobile phase was replaced with 60% 5 mM TBA in Methanol : 40% Aqueous IPCA Solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	4/14/08 Date	QC Specialist	4/19/08 Date	Reviewer	4/19/08 Date

Results:

Table 1. Diphacinone concentration in analyzed limpet samples.

Sample Description	Lab ID	Diphacinone Conc. (ppm)
Kalaupapa Reference Limpets	S080320-21	<MLOD
Mokapu 2/17 Limpet 1	S080320-22	<MLOD
Mokapu 2/17 Limpet 2	S080320-23	<MLOD
Mokapu 2/17 Limpet 3	S080320-24	<MLOD

Table 2. Quality Control Recovery for Diphacinone.

ID	Fortification Level (ppm)	% Recovery
QL 1	Blank	------
QL 2	Blank	------
QL 3	0.195	113
QL 4	0.201	101
QL 5	0.965	90.3
QL 6	0.975	101
Mean		101 ± 9.3

Kalaupapa Reference Limpets used for all QC samples (S080320-21)

Cc: Tom Primus
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April 30, 2008

Progress Report

Determination of Diphacinone Residues in Seawater Samples Following Aerial Broadcast of Bait on Mokapu Island, HI

By

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I. Introduction

Fish-flavored, pelletized cereal grain bait, containing the rodenticide diphacinone (2-diphenylacetyl-1, 3-indandione) at a concentration of 50 ppm, was aerial broadcast on Mokapu Island, HI on February 6, 2008 and February 12, 2008. Mokapu is a roughly 10 acre narrow-ridged island, rising 360 feet above sea level, approximately 0.7 miles off the north shore of Molokai in the Hawaiian Islands chain. Mokapu is a State Seabird Sanctuary and also supports 29 native plant species. Polynesian rats (*Rattus exulans*) are known to eat the eggs and chicks of three rare seabird species which nest on Mokapu: Wedge-tailed shearwaters (*Puffinus pacificus chlororhynchus*; 'ua'u kani), Red-tailed tropic birds (*Phaethon rubricauda rothschildi*; koa'e 'ula) and White-tailed tropic birds (*Phaethon lepturus dorotheae*; koa'e kea). Additionally, rats eat the seeds and shoots of two rare plant species; loulu lelo palms (*Pritchardia hillebrandii*) and ho'awa (*Pittosporum halophilum*). Ho'awa plants are represented by 14 individuals surviving in the wild with 11 of these individuals resident on Mokapu (1, 2). As part of the aerial broadcast study the following samples were collected on February 17, 2008: Seawater taken from six stations evenly spaced in adjacent waters around the island; Intertidal limpets (*Cellata exarata*; opihi) collected in the mid to high intertidal zones on the island; Fish (Blue-lined snapper (*Lutjanus kasmira*; ta'ape), hogfish (*Bodianus bilunulatus*; a'awa) and bridled triggerfish (*Sufflamen fraenatus*; hagi) collected from water adjacent to the west side of the island; and rodenticide bait samples left over following the aerial broadcast of the island.

This report summarizes data for diphacinone residues in the surface water grab samples collected within 30 feet of the eastern and western sides of Mokapu Island. Six 250mL samples were taken at each of the six stations. Three stations were located off the eastern shore and three off the western shore. Two samples from each site were provided for analysis. Additionally, reference seawater samples were collected on January 23, 2008 at Kalaupapa National Historical Park (Ka Laea Point) to serve as background and laboratory-fortified diphacinone positive controls.

II. Determination of Diphacinone Residues in Mokapu Seawater Samples

Sample Handling: Mokapu Island seawater samples were collected on 17 February, 2008 and packaged in 250mL sealed glass jars and stored refrigerated. They were shipped refrigerated, overnight to the Columbia Environmental Research Center (CERC) under official chain of custody on 17 March, 2008. Samples were logged in the CERC database, and kept refrigerated (4ºC) in the dark until analysis.

Summary of Analytical Method: A diagram of the steps involved in determination of diphacinone residues in the Mokapu seawater samples is shown in Figure 1. Accompanying the sets of seawater were the following quality control samples: an HLB-SPE blank, a reference seawater matrix blank, a reference seawater fortified matrix spike, a laboratory (Aqueous Oceanic Natural Sea Salt, Oceanic systems, Dallas, TX) control blank, and a laboratory (aqueous Oceanic Natural Sea Salt) fortified control matrix spike. A triplicate analysis of the Mokapu Station #6 seawater sample (CERC #: 42041) was also included.

Figure 1
Analytical Scheme for Diphacinone Isolation and Analysis from Seawater

1. Pre-condition HLB-SPE cartridge with 10mL acetonitrile.

2. Pre-condition cartridge with 10mL methanol.

3. Dry cartridge, under vacuum, for ~3 minutes.

4. Pre-condition cartridge with 10mL aqueous TBAH-IP reagent.

5. Pre-condition cartridge with 20mL 18mΩ Milli-Q water: (Cartridge remains wetted prior to sample application).

6. Apply 200mL water sample (100mL for triplicate, laboratory blank, and laboratory-fortified spike samples) to the cartridge at ~3mL/minute.

7. Rinse the flask, which contained the 200/100mL water subsample, with ~20mL 18mΩ Milli-Q water and pass the rinse through the cartridge at ~3mL/minute.

8. Wash the cartridge with 3mL 18mΩ Milli-Q water.

9. Dry the cartridge, under vacuum, for ~3 minutes.

10. Elute the cartridge with 13mL acetonitrile, collecting eluant in a 15mL amber culture tube – Fraction #1 (F1).

11. Recover any bound residues with 5mL 70:30 (methanol : aqueous TBAH-IP reagent; v:v), collecting the eluant in a separate 15mL amber culture tube – Fraction #2 (F2).

Materials: HLB-SPE = Water Oasis HLB (6cc X 500mg) SPE Cartridge (Waters Corp., Milford, MA)
Acetonitrile = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
Methanol = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
Aqueous TBAH-IP = 0.03M Tetrabutylammonium hydroxide (Sigma-Aldrich, St. Louis, MO) ion pair reagent in Milli-Q water, pH adjusted to 6.0 with 2N-o-phosphoric acid (Fisher Scientific-HPLC grade, Fair Lawn, NJ)
Milli-Q (18mΩ) water = Millipore Synergy UV (Millipore Corp., Bedford, MA)

Laboratory fortified samples were prepared in 200mL of Kalaupapa reference seawater and 100mL of aqueous Oceanic Natural Sea Salt solution by adding 2.5µg diphacinone (25µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT). The final concentration of the spiked samples were 0.013µg/mL for the 200mL reference sea water and 0.025µg/mL for the Oceanic Natural Sea Salt solution, respectively.

The acetonitrile fraction sample concentrates (F1) were evaporated to dryness using a nitrogen gas evaporator with a water bath temperature of <50ºC (Organomation N-EVAP, Berlin, MA). The residues were re-dissolved in 700µL methanol and 300µL of the aqueous TBAH-IP reagent was added to mimic the HPLC-PDA mobile phase. Prior to analysis, 1µg courmarin (10µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT) was added as an instrumental internal standard. The mobile phase recovery sample concentrates (F2) were analyzed directly. Prior to analysis, 5µg courmarin was added to the 5mL sample (50µL of a 100.8µg/mL solution in methanol).

The pH of the Mokapu Island samples were determined using a Mettler-Toledo Seven Easy pH meter (Schwerzenbach, Switzerland). The pH meter was calibrated with 4.00 and 7.00 buffer solutions (Fisher Scientific, Fair Lawn, NJ) prior to pH determinations of the remaining ~30mL of sample.

The following HPLC/PDA system was used for the quantification of diphacinone residues in the enriched seawater extracts:

Pump:	Thermo-Finnigan Surveyor LC Pump	
Autosampler:	Thermo-Finnigan Surveyor Autosampler	
Detector:	Thermo-Finnigan Photodiode Array Detector	
	Wavelength Scan:	230 nm to 400 nm
	Reference Wavelength:	400 nm
	Primary Wavelength:	286 nm
	Secondary Wavelengths:	314nm and 326 nm
Controller:	PC with Excalibur Software	
Column:	Phenomenex Luna C18(2) 100Å, 3µ (150 X 2.00mm)	
Guard Column:	Phenomenex Security Guard C18 Guard Cartridge	
Mobile Phase:	A: 70:30 (v:v) Methanol : 0.03M Tetrabutylammonium hydroxide at pH 6.0 with 2N-Phosphoric Acid in 18 mΩ Water	
Flow Parameters:	Isocratic 100% A	
Flow Rate:	0.20 mL/minute	
Injection Volume:	20µL	

The primary wavelength for the instrumental internal standard, courmarin, was 276nm with a secondary wavelength of 312nm.

A calibration curve was prepared to contain 0.01µg/mL to 5µg/mL diphacinone and courmarin using the AccuStandard solutions with appropriate dilutions in mobile phase.

III. Results and Discussion

Concentrations of diphacinone residues in Mokapu Island seawater samples were below the limit of detection (LOD) of 0.018µg/mL. The LOD was set at 3 times background area and the limit of quantitation (LOQ) was set at 10 times backround area (3) for the background associated with fraction #1. Fraction #1 contained >98% of the spiked diphacinone residues in the laboratory fortified seawater samples. The LOD and LOQ for the method were: 0.018µg/mL and 0.061µg/mL, respectively. The instrumental internal standard (courmarin) recoveries for the HPLC sample analyses were 96% to 108%.

Table 1
Summary of Mokapu Island Seawater Analysis:

Station	CERC #	pH	Diphacinone (µg/mL)
1	42036	8.20	<0.018
2	42037	8.17	<0.018
3	42038	8.10	<0.018
4	42039	8.21	<0.018
5	42040	8.16	<0.018
6-Rep1	42041	8.17	<0.018
6-Rep2	-------	-----	<0.018
6-Rep3	-------	-----	<0.018

The isolation, concentration and HPLC/PDA method performed well throughout the residue analysis as summarized in Table 2. The efficiency of extraction, as monitored by fortified reference matrix and fortified control matrix, was 86% - 88%.

Table 2
Method Recoveries of Diphacinone in Various Fortified Sample Matrices

Identification	CERC #	pH	Diphacinone Recovery
Reference Blank	42033	8.10	n/a
Reference Spike	42033	8.11	86%
Control Blank	n/a	7.96	n/a
Control Spike	n/a	-----	88%
HLB-SPE Blank	n/a	n/a	n/a
HPLC Blank	n/a	n/a	n/a

As a confirmation of peak identity, the retention times, spectra and total scan : 286nm : 314nm : 326nm area ratios were compared to known diphacinone standards (Table 3). The retention times and spectra for the samples with <LOD diphacinone concentrations did not match the diphacinone standards. Additionally, the area ratios of the total scan,

primary quantitation wavelength and the secondary quantitation wavelengths did not correspond to known diphacinone standards

Table 3
Confirmation of Diphacinone Residues Based on Area Ratios

Standards:

Concentration	Total Scan	286nm	314nm	326nm
0.01µg/mL	1	4	2	2
0.05µg/mL	1	4	2	2
0.10µg/mL	1	4	2	2
0.50µg/mL	1	4	2	2
1.0µg/mL	1	4	2	2
5.0µg/mL	1	4	2	2
Spike Mock-1	1	4	2	2
Spike Mock-2	1	4	2	2

Samples:

Identification	Fraction	Total Scan	286nm	314nm	326nm
42033-Blank	F1	1	2	1	1
	F2	1	6	3	1
42033-Spike	F1	1	4	2	2
	F2	1	4	2	2
Control-Blank	F1	nd	nd	nd	nd
	F2	1	3	1	nd
Control-Spike	F1	1	4	2	2
	F2	1	4	2	2
HLB-SPE Blank	F1	nd	nd	nd	nd
	F2	1	4	nd	nd
42036	F1	1	0.4	0.1	0.1
	F2	1	2	1	1
42037	F1	1	0.4	nd	nd
	F2	1	4	1	nd
42038	F1	1	0.4	0.1	0.1
	F2	1	4	2	1
42039	F1	1	0.5	0.1	0.1
	F2	1	6	2	nd
42040	F1	1	0.5	0.1	0.1
	F2	1	5	2	nd
42041 (Average)	F1	1	0.5	nd	0.1
	F2	1	4	2	nd

nd = Not Detected

V. Storage Stability

Diphacinone has a water solubility of 30ppm and is subject to hydrolysis at pH 5. However, it is stable to hydrolysis at pH 7 and pH 9 (4). The Mokapu samples were stored for 53 days, refrigerated and in the dark, after collection and prior to analysis. The samples were collected on 17 February, 2008, shipped to CERC on 17 March, 2008 and HLB-SPE extracted on 10 April, 2008 (following method development and validation investigations). To determine the storage stability of diphacinone residues in seawater, 200mL of a Kalaupapa reference seawater sample (pH 8.1) was fortified with ~5µg total diphacinone. Additionally, a Milli-Q water sample (200mL) was also fortified with ~5µg total diphacinone. The two fortified samples were returned to refrigerated storage to mimic the holding times for the Mokapu samples. The storage stability samples are scheduled to be analyzed on 20 May, 2008.

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May 16, 2008

Progress Report

Determination of Diphacinone Residues in Fish Samples Following Aerial Broadcast of Bait on Mokapu Island, HI

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I. Introduction

Fish-flavored, pelletized cereal grain bait, containing the rodenticide diphacinone (2-diphenylacetyl-1, 3-indandione) at a concentration of 50 ppm, was aerial broadcast on Mokapu Island, HI on February 6, 2008 and February 12, 2008. Mokapu is a roughly 10 acre narrow-ridged island, rising 360 feet above sea level, approximately 0.7 miles off the north shore of Molokai in the Hawaiian Islands chain. Mokapu is a State Seabird Sanctuary and also supports 29 native plant species. Polynesian rats (*Rattus exulans*) are known to eat the eggs and chicks of three rare seabird species which nest on Mokapu: Wedge-tailed shearwaters (*Puffinus pacificus chlororhynchus*; 'ua'u kani), Red-tailed tropic birds (*Phaethon rubricauda rothschildi*; koa'e 'ula) and White-tailed tropic birds (*Phaethon lepturus dorotheae*; koa'e kea). Additionally, rats eat the seeds and shoots of two rare plant species; loulu lelo palms (*Pritchardia hillebrandii*) and ho'awa (*Pittosporum halophilum*). Ho'awa plants are represented by 14 individuals surviving in the wild with 11 of these individuals resident on Mokapu (1, 2). As part of the aerial broadcast study the following samples were collected on February 17, 2008: Seawater taken from six stations evenly spaced in adjacent waters around the island; Intertidal limpets (*Cellata exarata*; opihi) collected in the mid to high intertidal zones on the island; Fish (Blue-lined snapper (*Lutjanus kasmira*; ta'ape), hogfish (*Bodianus bilunulatus*; a'awa) and bridled triggerfish (*Sufflamen fraenatus*; hagi) collected from water adjacent to the west side of the island; and rodenticide bait samples left over following the aerial broadcast of the island.

This report summarizes data for diphacinone residues in the fish samples collected from Mokapu Island following the aerial broadcast of diphacinone bait. Fish samples were collected within 50 feet of the west side of the island. These included 4 blue-lined snapper (ta'ape; *Lutjanus kasmira*), 1 hogfish (hagi; *Bodianus bilunulatus*) and 1 bridled triggerfish (hagi; *Sufflamen fraenatus*). The fish were processed into skinless fillets with the remainder of the carcass discarded. Each fillet was divided into three equal portions, with one portion of each fish provided for analysis. Reference fish was purchased commercially at an Oahu market on March 17, 2008. Four blue-lined snappers were processed into fillets and an individual portion of each fish was provided for laboratory control samples.

II. Determination of Diphacinone Residues in Mokapu Fish Samples

Sample Handling: Mokapu Island fish samples were collected on 17 February, 2008 and packaged in aluminum foil packets sealed in zip-lock bags and stored frozen. They were shipped frozen, overnight to the Columbia Environmental Research Center (CERC) under official chain of custody on 17 March, 2008. Samples were logged in the CERC database, and kept frozen (-20ºC) in the dark until analysis.

Summary of Analytical Method: The steps involved in determination of diphacinone residues in the Mokapu fish samples is outlined below. Accompanying the set of fish samples were the following quality control samples: a procedural blank (PB), a procedural spike (PS), a reference ta'ape matrix blank (TMB), and a reference ta'ape

matrix spike (TMS). Additionally, triplicate analyses of the Mokapu Station #1-B ta'ape individual sample (CERC #: 42046) were also included.

Analytical Scheme for Diphacinone Isolation and Analysis from Fish

1. Homogenization of fish fillet samples with commercial blender, using stainless steel blender blades, in 4oz glass jar.
2. Dehydration of ~5g of fish filet sample, with anhydrous sodium sulfate (~5 times sample weight), in 4oz glass jar. Samples allowed to dehydrate for at least 2 hours with periodic mixing using a stainless steel spatula.
3. Dehydrated samples blended to fine, free-flowing powder using a commercial blender with stainless steel blades. Matrix transferred to individual 2cm ID extraction column with 20mL acetonitrile rinse of dehydration sample jar. (Note: Extraction column consists of Teflon® stopcock, glass wool, ~1cm layer of Na_2SO_4, sample matrix bed and ~1cm layer of Na_2SO_4 in ascending order). A 500mL boiling flask placed below column to collect effluent from extraction procedure.
4. Rinse solvent level allowed to descend to upper Na_2SO_4 layer and flow stopped. The saturated matrix beds allowed to stand for at least 1 hour prior to further extraction. (Note: Laboratory-fortified samples were spiked prior to rinse solvent application).
5. Acetonitrile extraction solvent (150mL) was added and flow rate adjusted to ~2mL/minute. Once the first extraction solvent level reached the upper Na_2SO_4 layer, an additional 100mL extraction solvent was passed through the column. The solvent was allowed to drain completely from the matrix bed.
6. The sample extracts were rotary evaporated to ~3mL under ~20inHg vacuum and a water bath temperature of <50ºC. The concentrated extracts were transferred to 15mL amber culture tubes with triplicate ~2mL sequential acetonitrile rinses.
7. The extracts were evaporated, under a gentle nitrogen gas stream, to 2mL.
8. Removal of co-extracted interferences was facilitated with C18(EC)-SPE cartridges. The SPE cartridges were pre-conditioned with: 10mL methanol; followed by 10mL of aqueous tetrabutyl ammonium hydroxide (TBAH-IP); were dried under vacuum for 1 minute; and finally 10mL acetonitrile. Care was taken to keep the SPE matrix saturated with acetonitrile prior to sample application.
9. The 2mL sample concentrates were applied to the SPE cartridge and flow was established via vacuum at ~2mL/minute. The sample tube was rinsed with three 1mL acetonitrile washes and applied to the SPE once the previous liquid layer had descended into the SPE matrix bed. The SPE was eluted with the remainder of the acetonitrile to recover the analyte of interest. (Note: Total elution volume was 10mL, or 3 X 1mL + 7mL acetonitrile).

Materials: Acetonitrile = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
C18(EC)-SPE = IST-Isolute C18 (End-Capped) (6cc X 500mg) SPE Cartridge (International Sorbent Technology, Mid Glamorgan, UK)
Methanol = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
Aqueous TBAH-IP = 0.03M Tetrabutylammonium hydroxide (Sigma-Aldrich, St. Louis, MO) ion pair reagent in Milli-Q water, pH adjusted to 6.0 with 2N-o-phosphoric acid (Fisher Scientific-HPLC grade, Fair Lawn, NJ)
Milli-Q (18mΩ) water = Millipore Synergy UV (Millipore Corp., Bedford, MA)

Laboratory fortified samples were prepared in ~5g of reference ta'ape and Na_2SO_4 blank matrix by adding 2.5µg diphacinone (25µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT) to the dehydrated reference material and Na_2SO_4 , respectively. The final concentration of the spiked samples were 0.50µg/g.

The acetonitrile SPE concentrates were evaporated to dryness using a nitrogen gas evaporator with a water bath temperature of <50ºC (Organomation N-EVAP, Berlin, MA). The residues were re-dissolved in 700µL methanol and 300µL of the aqueous TBAH-IP reagent was added to mimic the HPLC-PDA mobile phase. Prior to analysis, 1µg coumarin (10µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT) was added as an instrumental internal standard.

The following HPLC/PDA system was used for the quantification of diphacinone residues in the enriched fish tissue extracts:

Pump:	Thermo-Finnigan Surveyor LC Pump	
Autosampler:	Thermo-Finnigan Surveyor Autosampler	
Detector:	Thermo-Finnigan Photodiode Array Detector	
	Wavelength Scan:	230 nm to 400 nm
	Reference Wavelength:	400 nm
	Primary Wavelength:	286 nm
	Secondary Wavelengths:	314nm and 326 nm
Controller:	PC with Excalibur Software	
Column:	Phenomenex Luna C18(2) 100Å, 3µ (150 X 2.00mm)	
Guard Column:	Phenomenex Security Guard C18 Guard Cartridge	
Mobile Phase:	A: 70:30 (v:v) Methanol : 0.03M Tetrabutylammonium hydroxide at pH 6.0 with 2N-Phosphoric Acid in 18 mΩ Water	
Flow Parameters:	Isocratic 100% A	
Flow Rate:	0.20 mL/minute	
Injection Volume:	20µL	

The primary wavelength for the instrumental internal standard, coumarin, was 276nm with a secondary wavelength of 312nm.

A calibration curve was prepared to contain 0.01µg/mL to 5µg/mL diphacinone and coumarin using the AccuStandard solutions with appropriate dilutions in mobile phase.

III. Results and Discussion

Concentrations of diphacinone residues in Mokapu Island fish samples were below the limit of detection (LOD) of 0.010µg/g. The LOD was set at 3 times background area and the limit of quantitation (LOQ) was set at 10 times backround area (3) for the background associated with a reference ta'ape sample. The LOD and LOQ for the method were: 0.010µg/g and 0.034µg/g, respectively. The instrumental internal standard (coumarin) recoveries for the HPLC sample analyses were 96% to 104%.

Table 1
Summary of Mokapu Island Fish Fillet Analysis:

Station	CERC #	Species	Diphacinone (µg/g)
1-A	42045	ta'ape	<0.010
1-B Rep1	42046	ta'ape	<0.010
1-B Rep2			<0.010
1-B Rep3			<0.010
1-C	42047	ta'ape	<0.010
1-D	42048	ta'ape	<0.010
1-E	42049	a'awa	<0.010
1-F	42050	hagi	<0.010

Note: Concentrations (µg/g) based on sample wet weight.

The isolation, concentration and HPLC/PDA method performed well throughout the residue analysis as summarized in Table 2. The efficiency of extraction, as monitored by fortified reference matrix and fortified control matrix, was 100% - 102%. No diphacinone residues were found in the procedural of reference ta'ape blanks.

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May 22, 2008

Progress Report

Determination of Diphacinone Residues in Limpets Following Aerial Broadcast of Bait on Mokapu Island, HI

Stability of Diphacinone in Fortified Reference Seawater Samples after 53 Days Storage

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I. Introduction

Fish-flavored, pelletized cereal grain bait, containing the rodenticide diphacinone (2-diphenylacetyl-1, 3-indandione) at a concentration of 50 ppm, was aerial broadcast on Mokapu Island, HI on February 6, 2008 and February 12, 2008. Mokapu is a roughly 10 acre narrow-ridged island, rising 360 feet above sea level, approximately 0.7 miles off the north shore of Molokai in the Hawaiian Islands chain. Mokapu is a State Seabird Sanctuary and also supports 29 native plant species. Polynesian rats (*Rattus exulans*) are known to eat the eggs and chicks of three rare seabird species which nest on Mokapu: Wedge-tailed shearwaters (*Puffinus pacificus chlororhynchus*; 'ua'u kani), Red-tailed tropic birds (*Phaethon rubricauda rothschildi*; koa'e 'ula) and White-tailed tropic birds (*Phaethon lepturus dorotheae*; koa'e kea). Additionally, rats eat the seeds and shoots of two rare plant species; loulu lelo palms (*Pritchardia hillebrandii*) and ho'awa (*Pittosporum halophilum*). Ho'awa plants are represented by 14 individuals surviving in the wild with 11 of these individuals resident on Mokapu (1, 2). As part of the aerial broadcast study the following samples were collected on February 17, 2008: Seawater taken from six stations evenly spaced in adjacent waters around the island; Intertidal limpets (*Cellata exarata*; opihi) collected in the mid to high intertidal zones on the island; Fish (Blue-lined snapper (*Lutjanus kasmira*; ta'ape), hogfish (*Bodianus bilunulatus*; a'awa) and bridled triggerfish (*Sufflamen fraenatus*; hagi) collected from water adjacent to the west side of the island; and rodenticide bait samples left over following the aerial broadcast of the island.

This report summarizes data for diphacinone residues in the limpet samples collected from Mokapu Island following the aerial broadcast of diphacinone bait. Limpets were collected from three stations (Station #1 on the east side of the island and Station #2 and #3 on the west side of the island) on the island. A total of 40 limpets were collected and composited into three individuals from each site. Additionally, reference limpet samples were collected on January 23, 2008 at Kalaupapa National Historical Park (Ka Laea Point) to serve as background and laboratory-fortified diphacinone positive controls.

II. Determination of Diphacinone Residues in Mokapu Limpet Samples

Sample Handling: Mokapu Island limpet samples were collected on 17 February, 2008 and packaged in aluminum foil packets sealed in zip-lock bags and stored frozen. The samples were shipped frozen, overnight to the Columbia Environmental Research Center (CERC) under official chain of custody on 17 March, 2008. Samples were logged in the CERC database, and kept frozen (-20ºC) in the dark until analysis.

Summary of Analytical Method: The steps involved in determination of diphacinone residues in the Mokapu limpet samples are shown below. Accompanying the limpets were the following quality control samples: a procedural blank (PB), a procedural spike (PS), a reference limpet matrix blank (LMB), and a reference limpet fortified matrix spike (LMS). Additionally, triplicate analyses of the Mokapu Station #3 limpet composite sample (CERC #: 42044) were also included.

Analytical Scheme for Diphacinone Isolation and Analysis from Limpets

1. Homogenization of limpet samples by hand pulverization in original sample jar, and with a commercial blender, using stainless steel blender blades, in 4oz glass jar.
2. Dehydration of ~3g of limpet sample, with anhydrous sodium sulfate (Na_2SO_4; ~10 times sample weight), in 4oz glass jar. Samples allowed to dehydrate for at least 2 hours with periodic mixing using a stainless steel spatula.
3. Dehydrated samples blended to fine, free-flowing powder using a commercial blender with stainless steel blades. Matrix transferred to individual 2cm ID extraction column with 20mL acetonitrile rinse of dehydration sample jar. Diphacinone laboratory-fortified samples were spiked prior to rinse solvent application to matrix bed. (Note: Extraction column consists of Teflon® stopcock, glass wool, ~1cm layer of Na_2SO_4, sample matrix bed and ~1cm layer of Na_2SO_4 in ascending order). A 500mL boiling flask placed below column to collect effluent from extraction procedure.
4. Rinse solvent level allowed to descend to upper Na_2SO_4 layer and flow stopped. The saturated matrix beds allowed to stand for at least 1 hour prior to further extraction.
5. The limpets were extracted with acetonitrile (150mL) at a flow rate of ~2mL/minute. Once the first extraction solvent level reached the upper Na_2SO_4 layer, an additional 100mL extraction solvent was passed through the column. The extraction solvent was allowed to drain completely from the matrix bed following final elution.
6. The sample extracts were rotary evaporated to ~3mL under ~20inHg vacuum and a water bath temperature of <50ºC. The concentrated extracts were transferred to 15mL amber culture tubes with triplicate ~2mL sequential acetonitrile rinses.
7. The extracts were evaporated, under a gentle nitrogen gas stream, to 2mL.
8. Removal of bulk co-extracted interferences was facilitated with C18(EC)-SPE cartridges. The SPE cartridges were pre-conditioned with: 10mL methanol; followed by 10mL of aqueous tetrabutyl ammonium hydroxide (TBAH-IP); were dried under vacuum for 1 minute; and finally 10mL acetonitrile. Care was taken to keep the SPE matrix saturated with acetonitrile prior to sample application.
9. The 2mL sample concentrates were applied to the SPE cartridge and flow was established via vacuum at ~2mL/minute. The sample tube was rinsed with three 1mL acetonitrile washes and applied to the SPE once the previous liquid layer had descended into the SPE matrix bed. The SPE was eluted with the remainder of the acetonitrile to recover the analyte of interest. (Note: Total elution volume was 10mL, or 3 X 1mL + 7mL acetonitrile).
10. The samples were nitrogen evaporated to dryness and reconstituted in 3mL dichloromethane (DCM). Residual lipoidal constituents were removed by size exclusion chromatography (SEC) using SX-3 biobeads (70g), in a glass column, and DCM mobile phase at a flow rate of 3.5mL/minute. The effluent

was collected, in a 125mL boiling flask, from 40 to 60 minutes into the sample run.

11. Samples rotary evaporated and transferred to 15mL culture tubes with DCM rinses of the boiling flasks.

Materials: Acetonitrile = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
C18(EC)-SPE = IST-Isolute C18 (End-Capped) (6cc X 500mg) SPE Cartridge (International Sorbent Technology, Mid Glamorgan, UK)
Methanol = Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)
Aqueous TBAH-IP = 0.03M Tetrabutylammonium hydroxide (Sigma-Aldrich, St. Louis, MO) ion pair reagent in Milli-Q water, pH adjusted to 6.0 with 2N-o-phosphoric acid (Fisher Scientific-HPLC grade, Fair Lawn, NJ)
Milli-Q (18mΩ) water = Millipore Synergy UV (Millipore Corp., Bedford, MA)
Dichloromethane= Fisher-OPTIMA grade, (Fisher Scientific, Fair Lawn, NJ)

Diphacinone fortified samples were prepared with ~3g of reference limpet and a Na_2SO_4 blank matrix by adding 2.5µg diphacinone (25µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT) to the dehydrated reference material and Na_2SO_4 , respectively.

The DCM sample concentrates were evaporated to dryness using a nitrogen gas evaporator with a water bath temperature of <50ºC (Organomation N-EVAP, Berlin, MA). The residues were re-dissolved in 700µL methanol and 300µL of the aqueous TBAH-IP reagent was added to mimic the HPLC-PDA mobile phase. Prior to analysis, 1µg coumarin (10µL of a 100.8µg/mL solution in methanol; AccuStandard, New Haven, CT) was added as an instrumental internal standard.

The following HPLC/PDA system was used for the quantification of diphacinone residues in the enriched seawater extracts:

Pump:	Thermo-Finnigan Surveyor LC Pump	
Autosampler:	Thermo-Finnigan Surveyor Autosampler	
Detector:	Thermo-Finnigan Photodiode Array Detector	
	Wavelength Scan:	230 nm to 400 nm
	Reference Wavelength:	400 nm
	Primary Wavelength:	286 nm
	Secondary Wavelengths:	314nm and 326 nm
Controller:	PC with Excalibur Software	
Column:	Phenomenex Luna C18(2) 100Å, 3µ (150 X 2.00mm)	
Guard Column:	Phenomenex Security Guard C18 Guard Cartridge	
Mobile Phase:	A: 70:30 (v:v) Methanol : 0.03M Tetrabutylammonium hydroxide at pH 6.0 with 2N-Phosphoric Acid in 18 mΩ Water	
Flow Parameters:	Isocratic 100% A	
Flow Rate:	0.20 mL/minute	
Injection Volume:	20µL	

The primary wavelength for the instrumental internal standard, coumarin, was 276nm with a secondary wavelength of 312nm.

A calibration curve was prepared to contain 0.01µg/mL to 5µg/mL diphacinone and coumarin using the AccuStandard solutions with appropriate dilutions in mobile phase.

III. Results and Discussion

Concentrations of diphacinone residues in Mokapu Island limpet samples were below the limit of detection (LOD) of 0.017µg/g. The LOD was set at 3 times background area and the limit of quantitation (LOQ) was set at 10 times background area response (4) of the reference blank limpet sample. The LOD and LOQ for the method were: 0.017µg/g and 0.056µg/g, respectively. The instrumental internal standard (coumarin) recoveries for the HPLC sample analyses were 99% to 101%.

Table 1
Summary of Mokapu Island Limpet Analysis:

Station	CERC #	Species	Diphacinone (µg/g)
1	42042	opihi	<0.017
2	42043	opihi	<0.017
3 Rep 1	42044-1	opihi	<0.017
3 Rep 2	42044-2	opihi	<0.017
3 Rep 3	42044-3	opihi	<0.017

The isolation, concentration and HPLC/PDA method performed well throughout the residue analysis as summarized in Table 2. The efficiency of extraction, as monitored by fortified procedural matrix and fortified reference limpet matrix are summarized below. Additionally, individual steps in the sample preparation procedure were monitored for diphacinone residue recoveries.

Table 2
Method Recoveries of Diphacinone in Various Fortified Sample Matrices

Identification	CERC #	Diphacinone Recovery
Procedural Blank	n/a	<0.017µg/g
Procedural Spike	n/a	102%
Reference Limpet Blank	42034	<0.017µg/g
Reference Limpet Spike	42034	102%
C18(EC) Spike	n/a	99%
SEC Spike	n/a	100%

V. Storage Stability: 53 Day Analysis

Diphacinone has a water solubility of 30ppm and is subject to hydrolysis at pH 5. However, it is stable to hydrolysis at pH 7 and pH 9 (5). The Mokapu samples were stored refrigerated and in the dark for 53 days after collection and prior to analysis. The samples were collected on 17 February, 2008, shipped to CERC on 17 March, 2008 and HLB-SPE extracted on 10 April, 2008 (following method development and validation investigations). To determine the storage stability of diphacinone residues in seawater, 200mL of a Kalaupapa reference seawater sample (pH 8.1) was fortified with ~5µg total diphacinone. The fortified sample was returned to refrigerated storage to mimic the holding times for the Mokapu samples. The storage stability sample was analyzed on 19 May, 2008 using the methods outlined in the progress report "Determination of Diphacinone Residues in Seawater Samples Following Aerial Broadcast of Bait on Mokapu Island, HI." dated 30 April, 2008. The recovery of diphacinone residues in the 53 day fortified reference seawater storage sample was 88%. Analysis of a diphacinone spiked Milli-Q water sample (matrix spike QC sample), prepared the day of analysis of the storage sample, showed a method recovery of 95%.
Correcting for the matrix spike method recovery, the storage stability sample retained 93% of the expected diphacinone residues over the 53 day period.

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