

United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center
PROTOCOL COVER PAGE

Study Title:	Assessment of bait density, bait availability, and non-target impacts during an aerial application of rodenticide to eliminate invasive rats on Desecheo Island, Puerto Rico
NWRC Study Director:	Aaron Shiels
Approved NWRC Project:	Methods and strategies to manage invasive species impacts to agriculture, natural resources, and human health and safety

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input checked="" type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device, impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Aaron Shiels  28 January 2016
Study Director: _____ Date: _____

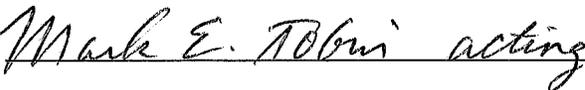
Position (check one):

- Biologist/Chemist/Technician
Supervisor signature required:
_____ Date _____ Res. Scientist Proj. Leader
- Research Scientist
- Project Leader
- Visiting Scientist: NWRC Representative/Contact: _____
- Student: NWRC Representative/Contact: _____

Concur: 
NWRC Research Project Leader Gary Witmer Date 3/14/2016

Review and Processing:
QAU: L. Auner Date 3/14/16

Concur: 
NWRC Assistant Director Mark E. Tobin Date 3/14/16

Approved: 
NWRC Director Mark E. Tobin acting Date 3/14/16

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
A. Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior wild animals in their natural habitat, as well as capturing and/or handling animals. <input checked="" type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
B. Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study require space, equipment or personnel from the NWRC Biological Laboratory System? If yes, date of consult with Laboratory Specialist:
C. Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required perm numbers and approval dates. ___DRNA (Puerto Rico) collection permit___ ___Not available yet___ ___applied Jan 20 2016___ Permit(s) description _____ Number _____ Date _____
D. National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
E. Regulatory Standard and Test Guidelines		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
F. Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
G. Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
H. Material Transfer Agreement /Chain of Custody		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc. to another facility)? If yes, complete the appropriate MTA or CoC Appendix .
I. Analytical Chemistry		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Aaron Shiels	NWRC Ft Collins	Study Director, Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Gary Witmer	NWRC Ft Collins	Project Leader, co-PI
Ken Gruver	WS Operations Puerto Rico	Cooperator/Consultant
Are Berentsen	NWRC Ft Collins	Technical Assistance/Consultant
Dave Goldade	NWRC Ft Collins	Technical Assistance/Consultant
Rachel Moulton	NWRC Ft Collins	Technical Assistance
Celeste Samra	NWRC Ft Collins	Technical Assistance
Kirsty Swinnerton	Island Conservation	Collaborator, Assist in field sampling
David Will	Island Conservation	Collaborator, Assist in field sampling
Gregg Howald	Island Conservation	Collaborator
Susan Silander	USFWS, Puerto Rico	Collaborator

2. Testing Facilities

Name	Address	Role in Study
NWRC Headquarters	4101 Laporte Ave, Ft. Collins CO 80521	Base for research & Chemical analysis

3. Sponsor

Name	Address	Contract No.
U.S. Fish & Wildlife Service	USFWS, Caribbean Islands NWR Complex, P.O. Box 510 Boqueron, PR 00622	NA

4. Schedule

Proposed Experimental Start Date: March 4, 2016
 Proposed Experimental Termination Date: September 30, 2016
 Proposed Study Completion/Archive Date: June 30, 2017

5. Background and Justification

Rats (*Rattus* spp.) have been introduced to many ecosystems worldwide and are among the most widespread and problematic invasive animals affecting islands (Towns et al. 2006). Through mostly unintentional introductions by humans, these rats now occupy > 80% of the major islands worldwide (Atkinson 1985; Towns 2009). As a consequence of their omnivorous diet and large incisor teeth, introduced rats are probably the invasive animals responsible for the greatest number of plant and animal extinctions on islands (Towns et al. 2006).

Dryland ecosystems are the most threatened terrestrial tropical ecosystems, and they are home to many rare, threatened, or endangered species. The island of Desecheo (1.5 km², or 0.6 miles² total land area), off the western shore of mainland Puerto Rico in the Caribbean, is a dryland ecosystem that is composed of mostly forest. It is a U.S. National Wildlife Refuge, and it is managed by the U.S. Fish and Wildlife Service (USFWS). There are several endemic reptile species on this island, and one endangered cactus. Marine and terrestrial birds are relatively uncommon to Desecheo, and it is thought that the low bird numbers are due to the infestation of the black rat, *Rattus rattus*. The black rat is the most common and destructive rat in tropical island forests (Shiels et al. 2014), and its suppression has resulted in major benefits to native and endangered species in tropical islands (e.g., Witmer et al. 2007; Pender et al. 2013). On Desecheo, *R. rattus* most-likely consumes native reptiles, birds, arthropods, and plants. The USFWS and Island Conservation (IC) had attempted to remove all rats from Desecheo in 2012 using aerial broadcast of the rodenticide Brodifacoum-25D Conservation. The motivation for such rat removal from Desecheo was to provide safe habitat for resident and non-resident birds, endemic reptiles, and native plants. Unfortunately, the rat removal operation of 2012 was unsuccessful at removing all rats, and since then rat populations have recovered to levels equivalent to those prior to the eradication attempt. After external review of the 2012 operation, IC and USFWS approached NWRC to change the Brodifacoum-25D Conservation label to increase the application rate and duration between applications. In November 2015, NWRC received approval from EPA to change the label for Brodifacoum-25D Conservation for use on Desecheo in 2016 (supplemental label EPA Reg. No. 56228-37). Key monitoring associated with the change in label for the 2016 Desecheo rat eradication is a requirement.

As with any projects that use toxicant bait, we expect that there will be some negative effects to non-target organisms (e.g., see Masuda et al. 2014; Pitt et al. 2015). Justification for proceeding with such a control tool that harms some non-target species is that the longer-term effects of removing rats will provide greater benefit to the native species and habitat that goes beyond the number (and types) of non-target mortalities. Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, there are no expected negative impacts to threatened or endangered species as a result of this rat eradication; there is one cactus species (*Harrisia portoricensis*; higo chumbo) listed as ESA threatened. Expected non-target impacts may include some species being affected by eating the bait directly or consuming any animal that has consumed the toxicant. On Palmyra Atoll where eradication of *R. rattus* was deemed a success by applying 4-8 times the brodifacoum stated on the original label, there were 84 carcasses of fish, birds, lizards, and invertebrates that were recovered dead shortly after the rodenticide applications (Pitt et al. 2015). Of the subset of these animals sampled, 84.3% had brodifacoum residues (Pitt et al. 2015), thus demonstrating the range of non-targets and multiple levels of the food web that can be affected by island-wide rat eradications that use brodifacoum. Marine biota (e.g., fish, mussels) can also accumulate residues of brodifacoum after rat eradications, and this was recently documented on an offshore island in New Zealand that had brodifacoum bait applied to eliminate rats (Masuda et al. 2015). In a literature review of studies measuring marine biotic exposure to brodifacoum, <6% of the fish and marine invertebrates sampled had brodifacoum residues (Masuda et al. 2015). Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, the terrestrial biota, particularly the lizard community, is expected to suffer from direct or indirect (via insect or rat carcass) consumption of the brodifacoum bait in the 2016 rat eradication attempt. By contrast, the marine and bird community is expected to suffer minimally from this operation given the few resident marine and terrestrial birds currently at Desecheo (K. Swinnerton, Island Conservation, personal communication) and the relatively few marine animals suffering brodifacoum exposure in other similar brodifacoum-based operations (Masuda et al. 2015).

The goal of USFWS and IC for Desecheo is to successfully remove all rats from the island in 2016. The operation will consist of USFWS and IC taking charge of the operation, and therefore facilitating the application of Brodifacoum-25D according to supplemental label (see below). While USFWS and IC will be conducting the bait application, NWRC will be conducting key associated monitoring for the project. For objectivity and best practice

procedures, the agency leading the operational aspects of the study should be different than those leading in the monitoring (Pitt et al. 2015). NWRC will not be poisoning animals. In this NWRC study protocol, the details of the monitoring portion of the study are outlined. The overall objectives for this study are to: 1) determine the distribution and density of bait from the aerial application of Brodifacoum-25D, 2) conduct the bait availability monitoring by revisiting plots and using motion cameras to determine animals removing or consuming bait, and 3) document the non-target effects through carcass searches and analysis of brodifacoum residues throughout the consumer food web, with concentration on the lizard community.

6. Related Protocols

QA-1736 Evaluation of candidate wildlife biomarkers to assess bait acceptance by mice (*Mus musculus*): comparison of biomarker detection and retention, and bait palatability.

QA-1605 A test of the efficacy of two commercial diphacinone baits on roof rats from Egmont Key, Florida.

QA-1875 Palmyra Atoll rainforest restoration project: rat eradication monitoring plan for alternatives B and C (Aerial broadcast of 25W).

QA-2240 Chemical tissue analysis of existing carcass specimens of Hawaiian raptors (Hawaiian hawk, short-eared owl, barn owl), Hawaiian goose, and Hawaiian hoary bat for presence of targeted rodenticide residues.

QA-2441 Wake Atoll fish tissue sampling and analysis three years after an island wide rodenticide application.

QA-2523 Assessment of a hand-broadcast rodenticide bait trial to control rats in the Waianae Mountains, Oahu

7. Assurance of Non-Duplication of Studies

Aside from the 2012 rat eradication attempt, which was unsuccessful, there have been no previous studies on Desecheo of an aerial broadcast of rodenticide to remove rats from the island. Similarly there has not been any monitoring of the rodenticide residues in the Desecheo food web. Island Conservation has produced an unpublished report of the bait availability monitoring and non-target impacts from the 2012 operation, which had a much lower (2-3 times) rodenticide bait application than what is proposed for 2016. There have been several monitoring studies (including those led by NWRC) from Pacific Islands (Palmyra and Wake) following aerial broadcast and whole-island rat eradication operations (e.g., QA-1875, QA-2441; Pitt et al. 2015), and many of these same monitoring methods will be used in this study.

On 28 January 2016, a literature search (covering years 1980-present) using Web of Science and GoogleScholar was conducted using combinations of keywords such as: brodifacoum rat control, *Rattus*, island, Puerto Rico, dry forest. There were no studies that duplicate the proposed study.

8. Objective/Hypotheses

The requirement of key monitoring associated with changes to the Brodifacoum 25-D label has formed our objectives and study goals for the NWRC portion of the 2016 Desecheo rat eradication attempt. Thus, the overall objectives for this study are to conduct: 1) bait application rate (density) monitoring, 2) bait availability monitoring, and 3) non-target species impacts.

We expect that bait will be applied according to supplemental label and it will stay available for at least 5-7 days after application, despite the presence and abundance of non-target invertebrates such as hermit crabs that will eat a portion of the toxic bait and render it unavailable to rats (Cuthbert et al. 2012). We further anticipate that invasive rats will be removed from Desecheo using the planned application of Brodifacoum-25D rodenticide, and that dead rats recovered after bait application will indeed contain elevated levels of brodifacoum residues. Post-application, detectable levels of brodifacoum residues will also be found in several parts of the non-target consumer food web, particularly within invertebrates that may be directly consuming the bait, and the lizard community that will probably gain brodifacoum exposure from consuming insects (all three lizard species that we will measure) and dead rats (ameivas) that suffered from the poison. We expect rats, crabs, and insects to be the animals most commonly

documented (using monitoring cameras) removing or consuming bait pellets, with much fewer incidences of bait consumption from lizards and birds. Based on the relatively low amounts of brodifacoum bait applied to Desecheo vs. Palmyra Atoll (e.g., Pitt et al. 2015), we expect minimal evidence of non-target mortality on Desecheo relative to Palmyra, yet higher non-target mortality in 2016 on Desecheo than found during the previous Desecheo rat eradication attempt of 2012 when 2-3 times less bait was applied (Island Conservation unpublished report). Finally, we expect there to be some legacy levels of brodifacoum residues in ameivas from the 2012 rat eradication attempt. Ameivas are predicted as the longest lived of the three lizards that are included in our study, and given that residues accumulate in vertebrate tissue, we only expect some of the ameivas captured pre-application to have detectable levels of brodifacoum residues.

An understanding of the effectiveness and non-target impacts of the brodifacoum baiting in the planned manner (i.e., according to supplemental label) will help future feasibility assessments of rat eradications from islands and the associated levels of risk to non-target species.

9. Methods/Procedures

Study Site: Desecheo (18°23'14"N, 67°28'19"W) is a small (1.5 km²) island approximately 27 km from the western shore of the main island of Puerto Rico. The terrain is rugged with karst limestone as parent material, and the peak elevation is 218 m. It is dominated by dry forest vegetation, and annual rainfall averages 1020 mm. The island is administered by the USFWS as a National Wildlife Refuge. *Rattus rattus* is abundant on Desecheo, and although non-native goats and non-native rhesus monkeys were once common to the island, they have been recently eradicated. Apparently Desecheo had one of the largest nesting colonies of brown boobies prior to rhesus monkeys being introduced to the island. The negative impacts of *Rattus rattus* to natural areas and native species on tropical islands is well known (Shiels 2010; Shiels and Drake 2011; Pender et al. 2013; Shiels et al. 2014). Removing rats from Desecheo is expected to restore Desecheo's natural ecosystems by: 1) increasing security for three endemic reptiles (Anole: *Anolis desecheensis*, Gecko: *Sphaerodactylus levinsi*, Ameiva: *Ameiva desecheensis*), 2) promoting recovery of the island's once abundant seabird breeding colonies, and 3) protecting the federally-listed higo chumbo cactus (*Harrisia portoricensis*).

Monitoring overview: NWRC will conduct key monitoring (outlined below) of the Desecheo 2016 rat eradication operation following a supplemental label for Brodifacoum-25D Conservation (EPA Reg. No. 56228-37). Although NWRC will direct and control this monitoring, field assistants will be provided to NWRC by USFWS and/or Island Conservation to increase monitoring efficiency and strengthen the interagency partnership. The details of each monitoring component are addressed below, and the summary of this study's monitoring includes the following:

- 1) Bait application rate (density) monitoring. The supplemental label allows two application periods planned 24 days apart. Each period consists of three applications of Brodifacoum-25D Conservation to prescribed areas of the island as directed on the supplemental label. Bait densities will be monitored in established plots throughout Desecheo Island to ensure bait was applied to the site at a rate of no greater than that allowed on the label, but at or above the minimum target ground application rate of 30 kg/ha.
- 2) Bait availability monitoring. Bait applied to the island will be monitored by revisiting plots at set intervals after each bait application period. Motion cameras will also monitor subsets of bait to determine the types of animals consuming or removing bait.
- 3) Non-target species monitoring. As with any project that uses toxicant bait, we expect that there will be some negative effects to non-target organisms (see Pitt et al. 2015). Expected non-target impacts may include some species being affected by eating the bait directly or consuming any animal that has consumed the toxicant. Our non-target monitoring at Desecheo will include: 1) conduct carcass searches before and after each bait application period, 2) assess non-target take of bait via motion-sensing monitoring cameras, and 3) assess the levels of brodifacoum residue in focal parts of the food web by sampling (pre- and post-bait application) rats, reptiles, and invertebrates. Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, there were few effects on birds, which, in addition to budget constraints, has enabled us to exclude birds from residue analysis.

Below are the detailed methods of each of the monitoring components for this study.

Bait application rate (density) monitoring: On the days of bait application, approximately 60 plots (each 1 m²; denoted with pin flags) will be visited throughout accessible areas of Desecheo that span a range of habitats. Due to many locations on Desecheo being dangerous to access, the locations of the monitoring plots will be placed at random around existing trails/transects. Upon visit, bait pellets inside the 1 m² area will be counted so that the mass per m² for the area can then be calculated. The operational goal is to provide a minimum of 30 kg/ha on the ground with allowances for overlapping swaths and additional baiting at zone overlaps and valley bottoms. The label limits each application period to the whole island to 5,793 kg. The island is roughly 134 ha (3D surface area), so the theoretical average application rate for the island is 44.2 kg/ha (40.6 kg/ha, or about 4 g/m², if bait stations are not included). The label states that the time between application periods is 24 days, but could be 17-66 days depending on weather and/or logistical considerations.

Bait availability monitoring: The 60 bait density plots will be used for bait availability monitoring (i.e., persistence and conditional change of bait pellets over 14 days). Beginning on the first day of bait application (following counts in plots for "Bait application rate (density) monitoring"), two bait pellets will be placed within 5 cm of the pin flag in each 1 m² plot. Monitoring the bait pellets will then commence each 1-3 days thereafter for a period of up to 14 days (earlier if bait disappears). Upon visitation, the number and condition of the two bait pellets per plot will be recorded. Partial baits will be estimated to the nearest 25%. The condition of the bait will be assessed as using the Craddock (2004) bait degradation scale, which incorporates details of wet, moldy, damaged, or intact bait. On approximately 12 of the bait fate monitoring plots, motion-sensing cameras will monitor the types of animals visiting the baits. Finally, at 15 additional randomly placed 1 m² plots, two bait pellets will be surrounded by ½" hardware cloth to exclude all rats (and other vertebrates) to better understand the longevity of bait pellets in the absence of rats. These camera-plots and vertebrate exclusion plots will be spread across habitats to account for environmental variation on the island.

Rat and non-target carcass searches: Carcass searches will occur before, during, and after each bait application period; these will involve walking transects while visually scanning the ground for any rat and other vertebrate carcasses in approximately a 2 m swath on either side of the trail/transect. Equal trail/transects will be walked/scanned during each of the carcass searching events (e.g., 'systematic sampling'), and the estimated schedule of approximately 6 periods (e.g., just prior to, during, and ~10-28 days after 1st and 2nd applications). If any rat or other vertebrate carcass is found, a gps location will be taken; the carcass will then be placed (with gloved hand) in a ziplock bag, labeled, and taken by NWRC staff for description, freeze-storage, and analysis at the NWRC chemistry laboratory. Any non-target animals found alive but obviously ill or distressed will be noted (location and behavior) but will not be euthanized because sickened animals can recover from the poison if they did not consume a lethal dose. However, rats, which are the target species for removal from the island, found to be ill or distressed will be euthanized by administering a sharp blow (hammer blow) to the head (AVMA 2013).

Non-target species monitoring: Non-target monitoring will include: 1) carcass searches before, during, and after bait application period (e.g., just prior to, during, and ~10-28 days after 1st and 2nd applications), and 2) assessment of the levels of brodifacoum residue in focal parts of the food web by sampling pre- and post-bait application. We have decided to focus our residue sampling on rats, lizards, and insects. Pre-application residue sampling will not only form baseline concentrations for which post-application monitoring should exceed if these animals are ingesting (directly or indirectly) brodifacoum, but it will also help establish if there are legacy residues residing in these animals from 2012 applications. Therefore, we are anticipating n = 20 samples pre-application for each of the three lizard species (i.e., anole, ameiva, and gecko), n = 15 rats, and n = 10 insect samples. For post-application, we are anticipating n = 20 samples of each of three lizard species, and n = 10 insect samples. This makes a total of 155 samples for brodifacoum residue analysis. Rat and other vertebrate (marine and terrestrial) carcasses recovered post-application will be stored and possibly analyzed at a later date (i.e., not included in this budget). Each of these animals are chosen for brodifacoum residue analysis because of their likelihood to directly consume the brodifacoum bait, or consume brodifacoum indirectly by either scavenging a carcass (e.g., ameiva) or ingesting insects that had consumed the rodenticide bait (e.g., anole, gecko).

Lizards will be hand- or noose-collected. Once captured they will be euthanized one of two ways: 1) exposing the lizards to isoflurane to immobilize and ideally overdose them, followed by cervical dislocation, or, if this isoflurane method does not prove effective, lizards will be euthanized by 2) manually applied blunt force trauma to the head (hammer blow to the head while restrained; AVMA 2013). Decapitation will not occur because of the need for whole body brodifacoum residue analysis. Rats will be collected with live traps rather than snap-traps because of the abundance of land crabs that may suffer from snap-traps. Traps will be baited with fresh chunks of coconut (each ca. 1 cm x 2 cm) or peanut butter, and pre-baiting with shredded coconut or peanut butter will take place 1-3 days prior to opening traps (Sugihara 1997; Shiels 2010). Traps will be checked at least daily (24 hours after setting); however whenever possible traps will be checked both in the morning and just before sunset. Rats will be euthanized by overdose of isoflurane followed by cervical dislocation. This method, recently practiced in QA2523, will be used for both euthanizing rats and lizards, and involves placing the trap containing the live rat (or the single lizard) into a 60 cm x 60 cm plastic ziplock bag with 1-2 cotton balls moistened with isoflurane (isoflurane moistened cotton balls will be placed in such a manner that the animals cannot make contact with them). Because the large ziplock bags are clear plastic, the animals can be easily monitored. Once unconscious, the rat (or lizard) will be removed from the bag and trap, and cervical dislocation administered. Insects (mixed species of likely lizard prey) will be hand, net, and pit-fall trapped; they will be killed by placing them in a ziplock bag and then in the freezer. Gloved hands will be used when collecting or handling any of these specimens, and all specimens (collected pre- and post-application) will be frozen and sent to NWRC chemistry lab where they will be analyzed for brodifacoum residue. Rats collected for chemistry analysis may also be sampled by NWRC staff for tissue that can be stored for genetic analysis; future genetic analysis may be necessary to determine if subsequent rats caught on Desecheo after the 2016 operation represent survivors or newly established rats on the island.

10. Experimental Design and Statistical Analyses

Bait density and availability: Bait density and availability will be summarized using descriptive statistics. A bait availability curve will be produced for both the rat/vertebrate exposed bait and the bait where rats/vertebrates were excluded. The types of animals visiting the baits (i.e. results of monitoring cameras) will be summarized according to time since bait application.

Rat and non-target searches: All vertebrates that are collected dead will be documented and will have associated gps points identifying their location when collected. The levels of brodifacoum residues will be summarized using descriptive statistics.

Non-target monitoring: Concentrations of brodifacoum residue assays will be reported in summary tables for the pre- and post-bait application periods and tabulated for concentrations found in each animal species.

Note that all rat presence/abundance monitoring before, during, and after bait application will be conducted by USFWS and IC.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
HS 004.00	Personal Protective Equipment
HS 008.00	Hazard Communication
FP 031.00	Live trapping, transportation to research facility, handling, and processing of <i>Rattus</i> spp.
AC/HI 007.00	Live trapping, handling, processing, and care of rats (<i>Rattus</i> spp.) at the Hawaii Field Station
FP 034.00	Recovery and handling of animals found dead during routine field activities
CH 011.00	Selected avian tissue preparation for chemical residue analysis
FP/HI 002.00	Anesthetizing, marking, and radio-tagging rats (<i>Rattus</i> spp.)

HS 013.03

Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Rat trapping (trap location, gender, body weight)
 - b. Lab chemistry data for brodifacoum residue
 - c. Field datasheets that include bait availability, bait density, carcasses from searches, and non-target collection
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-16			
A. Salary and Benefits (NWRC)	\$16,295			
B. Travel & per diem	\$8,964			
C. Supplies/bait	\$685			
D. Lab analysis	\$36,500			
Subtotal:	\$62,444			
APHIS Pooled Job Costs (11%)	\$6,869			
Indirect Costs (16.15%)	\$10,085			
TOTAL:	\$79,398			

14. Human Health and Safety

This study is not expected to cause adverse human safety issues. Adherence to all applicable SOPs will be mandatory and all personnel will be adequately trained to perform the required tasks.

Safety precautions for trapping will follow established protocols and the researchers will ensure safety by wearing PPE when handling live rat, lizards, and other animal carcasses. All investigators have appropriate training for the described tasks. The laboratory is equipped with safety gear and protocols for accidents. There is no risk to the public's health and safety—all materials and specimens will be contained in ziplock bags and stored frozen in Puerto Rico or Colorado.

15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study. Other individuals (e.g., USFWS and IC volunteers, helpers) assisting with the study will work together with trained staff.

Aaron Shiels, Study Director, NWRC Research Biologist, has extensive experience trapping rats, immobilizing rodents with isoflurane, working with rodenticides, capturing, euthanizing, and preserving invertebrates and lizards, and working in Puerto Rico and similar study locations.

Gary Witmer, Project Leader and Research Biologist, NWRC, has extensive experience trapping rats, immobilizing rodents with isoflurane, working with rodenticides, capturing, euthanizing, and preserving invertebrates and lizards, and working in similar Caribbean study locations.

Kirsty Swinnerton, Island Conservation, has extensive experience trapping vertebrates, especially rats and lizards, and working with rodenticides.

David Will, Island Conservation, has extensive experience trapping vertebrates, especially rats, and working with rodenticides.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

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Cuthbert, R.J., M. de La Brooke, and N. Torr. 2012. Overcoming hermit-crab interference during rodent-baiting operations: a case study from Henderson Island, South Pacific. *Wildlife Research* 39: 70-77.

Masuda, B.M., P. Fisher, I.G. Jamieson. 2014. Anti-coagulant rodenticide brodifacoum detected in dead nestlings of an insectivorous passerines. *New Zealand Journal of Ecology* 38: 110-115.

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Shiels, A.B., W.C. Pitt, R.T. Sugihara, and G.W. Witmer. 2014. Biology and impacts of Pacific island invasive species. 11. *Rattus rattus*, the black rat (Rodentia: Muridae). *Pacific Science* 68: 145-184.

Towns, D.R., I.A.E. Atkinson, and C.H. Daugherty. 2006. Have the harmful effects of introduced rats on islands been exaggerated? *Biological Invasions* 8: 863-891.

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Witmer, G.W., F. Boyd, and Z. Hillis-Starr. 2007. The successful eradication of introduced roof rats (*Rattus rattus*) from Buck Island using diphacinone, followed by an irruption of house mice (*Mus musculus*). *Wildlife Research* 34: 108-115.

19. Appendices

Indicate none or check attached appendices:

- None
 - Animal Use Appendix
 - Analytical Chemistry Appendix
 - Column E Explanation
 - Material Transfer Agreement
 - Microbiological/Biohazardous Materials Formulation and Use Appendix
 - NEPA and ESA Appendix
 - Test, Control and Reference Material/Device Use Appendix
 - Other: (include appropriate title) _____
- Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal Description

1) Animals:

Species, subspecies (if applicable):

Black rat: *Rattus rattus*

Anole: *Anolis desechensis*

Gecko: *Sphaerodactylus levinsi*
Ameiva: *Ameiva desecheensis*

Breed, strain and substrain (if applicable): Wild-captured

Total Number and Sex: We anticipate live-trapping up to 15 adult rats during the study (approximate 50: 50 sex ratio) and prior to bait application. Individuals that do not meet criteria of body size (e.g. small juveniles) or other criteria (e.g. outside of targeted sex ratio) will be released. Once bait application begins, no rats will be captured but dead rats (that suffer from the toxic bait) will be collected, stored frozen, and a subset will be analyzed for brodifacoum residue. Although we do not know how many dead rat (or other vertebrate) carcasses we will find post-application, we estimate 15-30 carcasses will be recovered in an intact form for which we can send to NWRC chemistry lab for storage and possible brodifacoum analysis.

We anticipate 40 individuals (20 pre- and 20 post-application) of each lizard species (Anole, Gecko, Ameiva) will be captured using hand or noose capture.

Body weight range:

Based on previous trapping, black rats between 40-60 (juveniles) and 61-200 g (adults).

Geckos are 2-6 g.

Anoles are 6-15 g.

Ameivas are 8-29 g.

Age: We expect to catch all ages.

B. Rationale for involving animals, for appropriateness of species, and for numbers Provide justification why this study requires the use of animals, and for the numbers to be used.

1) Rationale for involving animals: Rats are the target species for this operation and their study is necessary in order to understand their fate after rodenticide application, as well as if any legacy effects of brodifacoum residues persisted from the 2012 brodifacoum application. Endemic lizards are common predators of insects at the site and are necessary to understand the indirect pathways of brodifacoum movement through the foodweb (e.g. insects eat rodenticide bait and lizards eat contaminated insects), and otherwise the risk posed to endemic island lizards from such rodenticide application. Lizard sampling is also necessary to determine if there are any legacy effects of brodifacoum residues that have persisted from the 2012 brodifacoum application.

2) Rationale for appropriateness of the species to be used:

Rats are introduced invasive pests in the islands of Puerto Rico and Caribbean region that negatively impact agriculture and native flora and fauna. Rats on Desecheo are known to eat the eggs, juveniles, and possibly adults of the three endemic lizards (gecko, anole, ameiva), and these

lizards represent the most-likely vertebrates to be negatively affected by the brodifacoum toxicant on Desecheo.

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):

This target sample size of rats (15 individuals live-trapped and euthanized prior to bait application) was established as a robust sample size for detecting remnant (legacy) brodifacoum residues from the 2012 rat eradication. Aside from the 15 rats live-trapped, we will collect rat carcasses during the many weeks following brodifacoum application. Although we do not know how many carcasses we will find, partly because land crabs will probably remove or consume some carcasses, we estimate 15-30 carcasses will be recovered in an intact form for which we can send to NWRC chemistry lab for possibly (future) brodifacoum analysis.

Target sample sizes of 20 individuals of each of the three lizard species prior to bait application, and 20 individuals of each lizard species following bait application was established based on our need to representatively sample across the whole island (multiple habitats) for each species while also keeping costs down for residue analysis. There are thousands of individuals of each lizard species that have been recorded on Desecheo, yet our restricted budget for residue analysis would not allow a greater sample size than 40 per species, and use of less samples was discussed and determined to be inadequate (e.g., potential for high proportion of false negatives during residue analysis).

C. Source

Free-ranging (wild) rats, anoles, geckos, and ameivas will be captured live from Desecheo Island, Puerto Rico.

D. Method of identification of animals

Each captured animal will be given a unique label on the ziplock bag for which it is stored in after being euthanized.

E. Trapping/Collecting

Rats will be captured with Hagaruma or Tomahawk live traps (SOP: FP/HI 002.00). Traps will be checked at least once daily, during morning hours. Lizards will be hand- or noose-captured.

F. Transport

G. Handling/restraint

Rats will be held in live-traps, anesthetized using isoflurane (SOP: FP/HI 002.00), and when unconscious with shallow breathing they will have cervical dislocation administered. Specifically, this method will involve placing the trap containing the live rat into a 60 cm x 60 cm plastic ziplock bag with 1-2 cotton balls moistened with isoflurane. Because the large ziplock bags are clear plastic, the animals can be easily monitored. Once unconscious (usually within 30-90 seconds), the rodent will be removed from the bag and trap, and euthanized by cervical dislocation.

Lizards will be restrained by hand or noose, and then immobilized by isoflurane to euthanize by isoflurane overdose followed by cervical dislocation (as described above for rats).

However, if the isoflurane method does not prove effective, then the lizards will be held/restrained by foot or hand and then a hammer blow to the head will be delivered while restrained (manually applied blunt force trauma to the head; AVMA 2013).

H. Quarantine
N/A

I. Housing/maintenance
N/A (none will be housed)

J. Dietary contaminant exposure
N/A

K. Disposition of animals

Carcasses of rats and lizards will be saved for brodifacoum residue analysis. Ill or injured animals captured will be euthanized by overdose of isoflurane followed by cervical dislocation (rats and lizards), or by manually applied blunt force trauma to the head (lizards). All animal carcasses beyond those that will be analyzed for residues will be disposed of (buried) on island or at the local landfill on mainland Puerto Rico.

L. Animal pain or distress

1) Consultation with Attending Veterinarian:
Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: Dr. Gordon Gathright

Date of Consultation: February 2, 2016

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

No

Yes If yes, continue with the following items.

a) Alternative procedures:
N/A

b) Sedatives, analgesics, or anesthetics or Column E Explanation:
N/A

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

N/A

M. Euthanasia

Once captured, lizards will be euthanized one of two ways: 1) exposing the lizards to isoflurane to immobilize and ideally overdose them, followed by cervical dislocation, or, if this isoflurane method does not prove effective, lizards will be euthanized by 2) manually applied blunt force trauma to the head (hammer blow to the head while restrained; AVMA 2013). Live-trapped or injured rats will be euthanized by overdose of isoflurane followed by cervical dislocation. Decapitation will not occur because of the need for whole body brodifacoum residue analysis.

N. IACUC Approval

Date of IACUC Approval Letter: 24 February 2016

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

A. This study qualifies for a Categorical Exclusion because:

- It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

This is a monitoring effort conducted in conjunction with the operational use of rodenticides by the partner agency. NWRC's role is in routine monitoring of rodent activity levels and food web residues of rodenticide use by our partners and not associated with our monitoring activities.

- It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

- A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- B) not cause contaminants to enter water bodies
- C) not adversely affect any federally protected species or critical habitat
- D) not cause bioaccumulation
- This study does not qualify for a Categorical Exclusion.

B. Will this activity occur anyway even without involvement by NWRC?

No

Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity. The USFWS is compelled to protect natural resources in this area. The operational use of rodenticides in this area would occur without the monitoring services to be provided by NWRC.

C. Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

The sampling procedure is not expected to produce any lasting impacts on target species (rats, gecko, anole, ameiva) populations. Although IC and USFWS goal is eradicate all rats from Desecheo, our rat sampling will occur just prior to these efforts and removing 15 individuals will not have any effect on the thousands of rats on the island. Similarly, there are thousands of individuals of each of the three target lizard species (K. Swinnerton, unpublished data), and therefore removing up to 40 individuals of each species from the island will not have any population-level impacts.

D. Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

An objective of this study is to determine the non-target effects from the brodifacoum bait application, with particular emphasis on the lizard community. There are few ground foraging birds that could be incidentally trapped in the Hagaruma or Tomahawk live traps; therefore this is an unlikely scenario. The study site is far from homes so pets will not be at the site. There are no larger nocturnal vertebrates, such as alien ungulates or feral cats, which could be captured in the rat live traps. Because lizard collection is by hand and noose, there is essentially no chance of accidentally catching a non-target species. All non-target species that are alive in the traps will be immediately released; all non-targets that are dead in the traps will be disposed of at the local Puerto Rico animal disposal site.

Effects on T&E species and eagles:

E. Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

No

Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Other:

Consultations:

F. Did you consult with a state or federal agency specifically on this action?

No

Yes If yes, describe the date/mode/contact person and outcome of this consultation:

G. Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

No, permission not needed because:

Yes

Other:

Analytical Chemistry Appendix

If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

A. Number of samples to be analyzed (by type):

A total of 155 samples, made up of:

120 lizards (40 anoles, 40 geckos, 40 ameivas); 15 rats; 20 insect samples, will be analyzed for brodifacoum residues at NWRC Chemistry Lab, Ft. Collins, Colorado. Additional samples from "carcass searches", which may include additional marine and terrestrial vertebrates, will be held for possible future brodifacoum analysis if funding becomes available.

B. Storage conditions (temperature, container type, light/dark, duration):

All samples will be shipped frozen in concordance with SOP HS 013.02 and they will remain frozen (- 20 degrees) at the testing facility at the NWRC facility in Fort Collins, CO until processed.

C. Method title and number:

This study will use the established methods of chemical residue analysis.

D. **ACP Leader approval:** _____ Dave Goldade _____ **Date:** __10 Nov 2015__
(attach email or letter of concurrence from Analytical Services Project Team Leader)

From: "Goldade, David A - APHIS" <David.A.Goldade@aphis.usda.gov>
Date: November 10, 2015 at 10:07:35 AM MST
To: "Shiels, Aaron B - APHIS" <Aaron.B.Shiels@aphis.usda.gov>
Subject: RE: Another rodenticide study

Aaron-

To do all the samples would be around \$42,000. If this is too much, we can talk about ways to reduce the costs. To do all but the fish would be around \$30,000. If you want to reduce costs, you could reduce the number of pre samples unless you suspect prior rodenticide exposures.

-Dave

=====
David A. Goldade, Supervisory Chemist
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone: [\(970\) 266-6080](tel:(970)266-6080)
Fax: [\(970\) 266-6089](tel:(970)266-6089)
E-mail: David.A.Goldade@aphis.usda.gov
=====

-----Original Message-----

From: Shiels, Aaron B - APHIS
Sent: Tuesday, November 10, 2015 9:59 AM
To: Goldade, David A - APHIS
Subject: Another rodenticide study

Hi Dave

I am working on a monitoring plan for Desecheo Island rat eradication--it is a steep and dry island off of mainland Puerto Rico. It will be funded by Island Conservation. I am already getting heat for my budget being too high but the chemistry is a key component.

At minimum I am thinking of the following for brodifacoum residue analysis (generally n=10 for pre- and n=10 for post)

-rat livers (n=20)
-lizard sp1 (n=20)
-lizard sp2 n=20
-fish sp1 n=20
-fish sp2 n=20
-invertebrate (probably cockroach) (n=20) -hermit crab (n=20)

Can you price that full set? The lizards might be too small to do just liver, but I would prefer just liver if we can. Ditto with the fish.

Secondly, can you price it without the fish included?

Thanks
Aaron

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code: Brodifacoum-25D Conservation: Pelletized Bait for Conservation Purposes
 - a) Concentration and purity: Brodifacoum: 0.0025% (active ingredient)
 - b) Source: Bell Labs
 - c) Batch number: (to be determined upon bait manufacture/arrival)

For non-standard materials, describe the material/device in detail and provide the name and location of the formulation laboratory or facility that will prepare the material.

B. Describe any control or reference materials/devices

As above, for each material provide the chemical, bait or device

- 1) name or code: Brodifacoum-25D Conservation: Pelletized Bait for Conservation Purposes
 - a) Concentration and purity: Brodifacoum: 0.0025% (active ingredient)
 - b) Source: Bell Labs
 - c) Batch number: (to be determined upon bait manufacture/arrival)

C. Carriers, mixtures and material preparation

This is the standard Brodifacoum-25D bait for which NWRC holds the label.

If materials are to be prepared by NWRC ACP Formulation Chemist, complete the following:

ACP Formulation Chemist Consultation: _____ Date: _____

D. Route of administration

Rodent consumption of bait pellets.

E. Dosage

Bait will be spread according to supplemental label Brodifacoum-25D Conservation (EPA Reg. No. 56228-37), at a rate of at least 30 kg/ha, but the theoretical average application rate for the island is 44.2 kg/ha (40.6 kg/ha if bait stations are not included). The supplemental label allows two application periods planned 24 days apart, but could be 17-66 days depending on weather and/or logistical considerations.

F. Test, control, and reference substance accountability

Test, control, and reference substance accountability will be according to SOP AD 012.03. The batch of Brodifacoum-25D bait will be subsampled, the subsampled bait will be labeled/documented and stored, then shipped and analyzed at the NWRC Analytical Chemistry

Lab according to SOP AD 012.03. Tracking numbers will be reported and documented as necessary.

TCRS tracking number(s): _____