

Mouse biomarker trial: South Farallon Islands

Trials to Examine Bait Acceptability, Consumption, and Non-Target
Exposure Risk



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1. INTRODUCTION

1.1 Purpose

The Farallon Islands provide critical habitat for seabirds and pinnipeds, and support some of the world's largest nesting seabird colonies including Ashy Storm-Petrel (*Oceanodroma homochroa*), Brandt's Cormorant (*Phalacrocorax penicillatus*) and Western Gull (*Larus occidentalis*). On the South Farallon Islands, which include two main islands- Southeast Farallon and West End Islands, introduced house mice (*Mus musculus*) appear to be directly and indirectly impacting the breeding success of burrow nesting seabirds (Ainley and Boekelhide 1990; Sydeman et al. 1998; Pyle 2001). The US Fish and Wildlife Service (USFWS) propose to eradicate house mice from the South Farallon Islands to improve habitat for nesting seabirds. The preferred technique for mouse eradication requires an aerial application of pelletized grain bait containing rodenticide across the island. Further background information and justification for this eradication are discussed in Howald et al. (2003) and the environmental impacts of this proposed action are analyzed in a draft environmental assessment (EA).

Removal of mice will result in biodiversity benefits for native flora and fauna on the Farallon Islands. Eradicating house mice from islands, however, has proven to be more challenging than removing rats from islands. While rats have been eradicated from 318 islands worldwide, only 20 successful mouse eradications have been documented (Howald et al. 2007). Furthermore, the failure rate of rat eradications is reported at 5percent, while in mouse eradications it is 19 percent (Howald et al. 2007). Despite a global review of mouse eradication attempts, no consistent explanations have been identified for this low rate of success (MacKay et al. 2007). However, compared to rats, it is known that mice are less susceptible to rodenticides, have a smaller home range and a more complex social structure, and feed somewhat sporadically from several locations rather than foraging on a regular, reliable food source (Macdonald and Fenn 1994). The behavioral and foraging differences between rats and mice indicate that to successfully remove mice from islands, a high standard of bait quality and availability to mice must be guaranteed. These standards can be better achieved by implementing a trial bait application prior to the proposed eradication to indicate any site-specific differences in mouse behavior, densities, or bait acceptability, which will help to refine standardized eradication methods for the local environment.

Prior to the proposed eradication, a field trial will be conducted to: i) validate the acceptability of proposed bait by house mice; ii) determine the rate of bait removal to extrapolate a target application rate

for the eradication; iii) determine the probability of eradication by using mice exposed to a biomarker from a non-toxic, biomarker-infused bait applied at the target application rate; and iv) evaluate what non-target species are at risk of rodenticide exposure during an eradication operation.

This proposal describes a field trial that will be conducted in fall 2009 to assess the efficacy of mouse eradication at the target application rate of a preferred bait (using a placebo replica infused with the non-toxic biomarker pyranine), and to monitor non-target species exposure to broadcast pellets.

1.2 Objectives

The field trials on the Farallon Islands are proposed to evaluate key factors related to the eradication to best inform a plan proceeding with a high likelihood of success. Specifically, the trial will be designed to address the following questions:

- i) At what density and phase of the reproductive cycle are mice during the proposed time period (fall) on the Farallon Islands?
- ii) Do mice demonstrate a preference for a non-toxic replica of the preferred bait, Brodifacoum-25D Conservation, to be used in the proposed eradication compared to naturally available food resources?
- iii) Based on rates of removal, at what density can bait be applied to persist for the duration of the target exposure window (10 days) for mice on the island?
- iv) At the target application rate will bait be delivered to all mice within the study areas?
- iv) What non-target species are at risk of rodenticide exposure during an eradication operation?

1.3 Impacts of house mouse to island ecosystems

The house mouse is among the most widespread of all mammals, a result of its close association with humans and the relative ease with which it can be transported and introduced to new locations. House mice are among the vertebrates considered to be “significant invasive species” on islands of the South

Pacific and Hawaii, having probably reached all inhabited islands in the Pacific as well as some uninhabited islands (Atkinson and Atkinson 2000). The resourcefulness of house mice is evident from their global distribution and broad habitat range including buildings, agricultural land, coastal regions, grasslands, salt marshes, deserts, forests and subantarctic areas (Efford et al. 1988; Triggs 1991; Atkinson and Atkinson 2000).

House mice eat a variety of seeds, fungi, insects, other small animals, reptiles and eggs of small birds, and their diet directly contributes to ecosystem-wide perturbations including effects on native fauna and flora (Rowe-Rowe et al. 1989; Crafford 1990; Amarasekare 1994; Cole et al. 2000). For example, Newman (1994) found that increased predation by house mice caused the capture rate for McGregor's skink (*Cyclodina macgregori*) to decline on Mana Island, New Zealand. After successful mouse eradication, the population of McGregor's skink, the gecko (*Hoplodactylus maculatus*), and the endemic giant cricket (*Deinacrida rugosa*) increased significantly. More recently, on Gough Island in the South Atlantic, house mice have been documented depredating large nestlings of Tristan Albatross *Diomedea dabbanena* and Atlantic Petrels *Pterodroma incerta*, and causing significantly reduced nesting success in these species (Wanless et al. 2007).

1.4 Project setting

The Farallon Islands are a small group of five islands located 51 km (32 miles) west of San Francisco. The total land area is 83 ha (211 acres) of which two islands, Southeast Farallon (31 ha) and West End, comprise the majority (44 ha). West End and Southeast Farallon Island (SEFI) are separated by a narrow gap known as the Jordan Channel. The islands are projections of a granitic ridge that rise from the sea floor, and the highest point is on SEFI at 105 m (343 ft).

During the 1800s, the Farallon Islands were exploited by seal hunters and egg collectors providing major sources of food for nearby San Francisco. In 1855 the U.S. Coast Guard established a lighthouse on SEFI and subsequently several keepers, their families and domestic animals lived there until 1965. It is likely that the house mouse was introduced to SEFI in the 1800s, along with domestic cats and rabbits (*Oryctolagus cuniculus*) during later settlement by lighthouse staff. The rabbits and cats were removed in 1974 but the house mice remain.

Owing to the overexploitation of the islands, in 1909 the North Farallon Islands were designated a national wildlife refuge (the Farallon Reservation), and in 1969 the South Farallon Islands were included

to create the Farallon National Wildlife Refuge. The islands are managed by the U.S. Fish and Wildlife Service, in conjunction with the PRBO Conservation Science and the U.S. Coastguard

1.5 Summary of knowledge of house mouse on the Farallon Islands

Over the past 200 years, the South Farallon Islands supported introduced rabbits, cats and house mice. Like rabbits and cats (which were successfully eradicated), house mice were introduced by previous human occupants of the island – before it became part of the Farallon National Wildlife Refuge in 1969. Information collected to date on the house mouse of the South Farallon indicates they:

1. Are distributed evenly on Southeast Farallon Island (SEFI) and have been observed on the West End Island (Irwin 2006).
2. Have not been observed on other islands in the chain (e.g., North or Middle Farallon Islands), nor are they suspected to occur on these islands, which have no history of human occupation.
3. Breed prolifically from April through November, and die off in equally large numbers from November through April (Irwin 2006).
4. Feed on native plants, invertebrates and seabirds (Jones and Golightly 2006; Irwin 2006; Ainley and Boekelheide 1990).

Trap studies carried out between 2001 and 2003 on SEFI suggest that mouse numbers peak between September and December reflected by a peak in trapping success (71%) in October, after which they decline to a minimum in April and May with only 2% trapping success in April (Irwin 2006). During the these three years, average trapping success was relatively low (25%-41%, mean 31%), a consideration for the trial study design when a reasonable sample size of mice trapped might be required.

2. METHODS

2.1 Mouse biology

2.1.1 *Relative abundance*

The relative abundance of mice will be assessed using mark-recapture surveys on a trapping grid. Sherman live traps (H.B. Sherman Traps, Tallahassee, Florida) will be arranged in a single 16 x 16 grid, with traps spaced at 10-m intervals. Traps will be lined with a small handful of polyester fiberfill for

thermal protection and baited with whole oats or peanut butter mixed with rolled oats. Traps will be checked the following morning as early as possible. Captured mice will be tagged using Size 1 (National Band and Tag Co., Newport, KY) ear tags, and age class, sex, weight, and reproductive status determined. For each mouse recaptured during subsequent trap sessions, the capture station and mouse ear tag number will be recorded. With a session equaling one trap night a minimum of five consecutive trap sessions will be conducted per survey. Sessions will be run at continuous intervals, with a minimum of three nights between repeated surveys. Mouse density will be approximated using the program CAPTURE.

As an additional index of abundance, capture rates will be calculated as (R) per trap nights (TN) (defined as one trap set per night) and corrected (C) by 1/2 for all sprung traps without a mouse catch (Nelson and Clark 1973) and notated as $R/C * TN$. This requires a trap status for each trap checked in the field as either “sprung” or “unsprung”. For “sprung” traps, evidence of a mouse capture or no obvious signs of capture will be recorded.

2.1.2 Movements and home range

Understanding the daily movement of mice is important to developing eradication operations specifically tailored towards the species. While mark-recapture methods could be used to assign home range size, these studies often underestimate home range size and movements in rodents, and baited traps can lure animals to areas where they might not naturally travel (Hall and Morrison, 1997). Therefore, we will radio collar and follow individuals to gain a better understanding of mouse home range and movements.

A subset ($n=20$) of captured mice will be fitted with a radio collar (1.0 g, M1400 Advanced Telemetry Systems, Isanti, MN) around the neck of the animal. Radio collars will transmit for approximately 4 weeks. Collared mice will be released at the location where they were captured and not located for ~12 hr to allow animals to assimilate back into their surroundings. After this initial release period, collared mice will be radio tracked using portable VHF receivers (Communication Specialists Model R1000) and a 2-element Yagi antenna. Mice will be monitored for at least 5 nights/week, 3 times/night with observation times spread across the nocturnal period. During each monitoring period the location of each signal fix or den site and the activity of the mouse (active or resting) will be recorded. The geographic location of each fix will be marked using a handheld GPS unit.

Mouse location points will be imported into ArcView (version 3.2; ESRI, Redlands, California), and the ArcView extension Animal Movements used to quantify summary statistics of radio collared animals.

The minimum convex polygon (MCP) and fixed-kernel home range estimates will be calculated for each animal.

2.2 Bait acceptability

Bait acceptability and preference will be evaluated in laboratory trials. A non-toxic replica of the preferred bait to be used during the proposed eradication will be tested for palatability in paired trials. Paired trials will test the preference of different bait size (3/16" diameter vs. 3/8" diameter pellet size), and test bait against naturally available food sources (*Claytonia* spp. or *Hordeum* spp. green plant material, and Coleopteran beetle or larvae) (Jones and Golightly 2006). Individual mice will be live-trapped and held in a field laboratory for a minimum of 24 hours pre-trial. Each mouse will be presented with paired food choices placed in random locations within the cage to reduce spatial selectivity. Trials will be scored by first bait/food type selected, with observations made for an additional period after choice to determine if animals switched to consuming the alternative bait/food type. Summary statistics of mouse preference for bait size and food type will be quantified for each trial.

2.3 Bait consumption

A bait consumption study will be conducted to quantify the rate of removal on the Farallon Islands. For an effective mouse eradication, bait must be on the ground and available to the target animal for a minimum of ten nights to ensure consumption of a lethal quantity. A trial bait application examining the rate of bait disappearance in the environment over time, using a non-toxic replica of the preferred bait will allow for the calibration of an optimal application rate that will ensure sufficient bait availability to mice for a period of ten days.

Bait consumption rates may vary on the island in relation to changes in mouse population density throughout the year, therefore bait uptake trials should occur at the same time of year as the proposed eradication. Ideally, an eradication would coincide with natural seasonal mouse population declines related to a depletion of food stuffs on the island. Based on mouse trap success rates from Southeast Farallon which fluctuate widely but relatively predictably through the year, from a peak in October (71%) to a low in April (2%), the fall months would be an ideal window for the eradication to maximize natural population declines (Irwin 2006).

2.3.1 Bait

The bait used during the bait consumption study will be a non-toxic replica of Brodifacoum-25D bait (Bell Laboratories Inc., Madison, WI, EPA Reg. No. 56228-37). 25D is a cereal-grain bait colored green and compressed into two bait sizes, 3/16" or 3/8" diameter pellets. The 25D bait formulation is an exact mimic of the preferred toxic bait to be used in the proposed eradication.

2.3.2 Bait broadcast and uptake

The non-toxic bait will be manually broadcast at a rate of 32 kg/ha using a method that mimics an aerial bait broadcast (Note: The application rate of 32 kg/ha is intentionally high to ensure that bait is available on the ground for the duration of the study, and does not reflect the application rate that would be applied in a proposed eradication.) Bait will be applied to a study area approximately 200 m², replicated in two areas using a 3/16" diameter bait in one area and 3/8" diameter in the other. In each study area, a team of hand-broadcasters will be spaced 10 m apart, and walk parallel transects across the width of the study area stopping every 10 m to uniformly apply bait in all four directions.

The consumption of bait within the study areas will be measured in fixed plots buffered at least 50 m from the edge of the baited area; exact distance to be determined from the outcome of the home range study. This buffer zone is needed to overcome the influence of animals outside of the treatment area on the bait uptake rate at the edges. Plots will be 50 m long x 1 m wide. Wire flags will be haphazardly scattered in each plot and a single bait pellet placed at the base of each flag during the application. Researchers will ensure that each plot has the number of pellets corresponding to the target application rate. Plots will be monitored every 24 hrs for ten days. Flags attending pellets will be left alone while flags without pellets will be removed and tallied.

The total number of bait pellets consumed from each plot will be used to extrapolate an application rate suitable for mouse eradication by aerial broadcast. The number of pellets remaining in each plot will be converted to kg/ha by multiplying the number of pellets remaining by the mean dry pellet weight, then dividing by the plot area (in ha). The consumption rate for each plot will be calculated by subtracting the remaining bait (kg/ha) from the target application rate. The mean and standard deviation of the consumption rate for all plots will be calculated and the upper 99% confidence interval for the sample mean will be used as the target application rate.

2.4 Biomarker bait trial

A biomarker bait study will be conducted to test the efficacy of the target application rate of Brodifacoum-25D Conservation, and to monitor exposure of non-target species to broadcasted bait. As closely as possible methods used during the biomarker trial will mimic those for the proposed aerial eradication in order to provide a relative index of eradication outcome.

2.4.1 Biomarker bait

The non-toxic formulation of Brodifacoum-25D bait will be infused with the non-toxic dye pyranine. Pyranine (also known as Solvent Green 7) is a commonly used dye found in commercially available products such as fluorescent marker pens, shampoos, soaps and cosmetics. It is also used to trace water-flows such as in plumbing systems, sewers and natural water-courses. It is non-hazardous, non-flammable and is not regulated as a hazardous material (refer to Appendix 1 for pyranine MSDS), is soluble in water and fluoresces under ultra-violet light. During bait palatability trials for *Rattus* sp. and *Mus* sp. on Macquarie Island (Australia) in 2005, pyranine was used in a cereal-based placebo bait, and under ultra-violet light was detected in animals' mouths, paws, anus and fur, as well as in feces and urine (K. Springer pers. comm.). Pyranine has also been used as a biomarker in trial rat eradications in New Zealand to investigate potential bait uptake in non-target birds on Macauley Island (Greene et. al 2002) and Little Barrier Island (Greene and Dilks 2004).

2.4.2 Mouse biomarker detection assay

A detection assay will be conducted prior to field-based biomarker trials to index detection level and persistence of biomarker sign in mice. These trials will be conducted in an approved laboratory prior to the field trial. Forty mice will be individually housed in plastic cages with wire lids and lined with sawdust for thermal protection. Mice will be acclimatized to the laboratory environment for a minimum of 24 hours, during which they will have free access to water and rodent feed.

In the retention study, mice will be randomly assigned to three non-toxic, biomarker bait exposure groups: <LD50, >LD50, and control. LD50 is defined as the amount of rodenticide bait that must be consumed in order to reach roughly 50% probability of mortality. House mice require approximately 1-2.6% of their bodyweight in a single dose to achieve acute oral toxicity of brodifacoum delivered at 20

ppm (Eason and Ogilvie 2009). Each bait exposure group will contain a minimum of 4 mice, with control treatment group containing only one mouse.

Mice in each group will receive a single feeding of a set number of non-toxic Brodifcaoum 25D Conservation bait pellets (infused with the biomarker pyranine; Bell Laboratories, Madison, WI) to achieve the assigned exposure. The number of pellets will be determined following validation of mean pellet mass (measured in grams), however should approximate 4, 8, and 0 pellets, respectively. Pellets will be weighed prior to feeding then placed in a receptacle and placed on the inside of the cage. Mice in the control group will be offered non-toxic pellets without biomarker. After all bait pellets are consumed by each mouse, they will be maintained on a diet of standard rodent feed. Mice in each exposure group will then be randomly assigned to a post bait consumption sampling period: 2 days, 4 days, 6 days, or 8 days. At each sampling period assigned mice will be euthanized (according to current UVMA standards) and sampled externally and internally for fluorescence using handheld ultraviolet lights. Externally the anus, mouth, and tail will be examined for external biomarker sign, while the alimentary tract (including stomach, intestines, and ceacum) will be inspected for internal sign of fluorescence. For each mouse examined the intensity of fluorescence will be qualitatively assigned to a rating scale.

<u>Score</u>	<u>Description</u>
-	No fluorescence detected
+	Fluorescence detected in limited intensity and/or area covered
++	Fluorescence detected in moderate intensity and/or area covered
+++	Fluorescence detected in high intensity and/or area covered

At the assigned termination day morphometric information, including body measurements, sex, and weight, will be collected for each mouse. For each exposure group the proportion of mice displaying fluorescence will be calculated for each sampling period. Maximum retention will be defined as the number of days post-feeding where fluorescence is not detected in < 80% of the study mice.

Results from the detection assay will be used as a baseline index of biomarker exposure during field-based trials.

2.4.3 Biomarker bait broadcast

Biomarker bait will be broadcast in two applications and replicated across two study areas on the island with 3/16” diameter bait applied in one area and 3/8” diameter in the other. During the first application

bait will be broadcast at the optimal application rate, as determined in the consumption trial (see section 2.3.2). The second application will occur ten days after the first, with bait applied at half the initial density. Each baited area will measure at least 200 x 200m. Bait application will follow the same standardized protocol described for the consumption trial, except during the second application bait will be broadcast on transects perpendicular to those of the first application.

2.4.4 Biomarker study area

The primary considerations for study sites for the bait uptake trials are:

- Areas that are sufficiently isolated from the consumption trial in space to ensure that the study species (mice and gulls) would not have access to residual bait
- Areas that have a comparatively high density of mice
- Areas that have a comparatively high density of roosting gulls (which will likely be the primary non-target bait consumer)
- Areas that can be traversed relatively easily on foot without disturbing sensitive resources such as seabird burrows or pinnipeds. To facilitate ease of implementation, a relatively flat and open area is required for treatment.
- Areas that provide sufficient ground area to provide meaningful bait uptake results from a “core” study area with a large baited “buffer” zone.

On preliminary investigation, the island areas identified on Southeast Farallon that appear to fit these criteria best are 1) the Marine Terrace area and 2) North Landing (Figure 1). The proposed areas are approximately mapped in Figure 1, however exact study locations will be determined together with FWS or PRBO staff to reduce impact to sensitive bird areas.

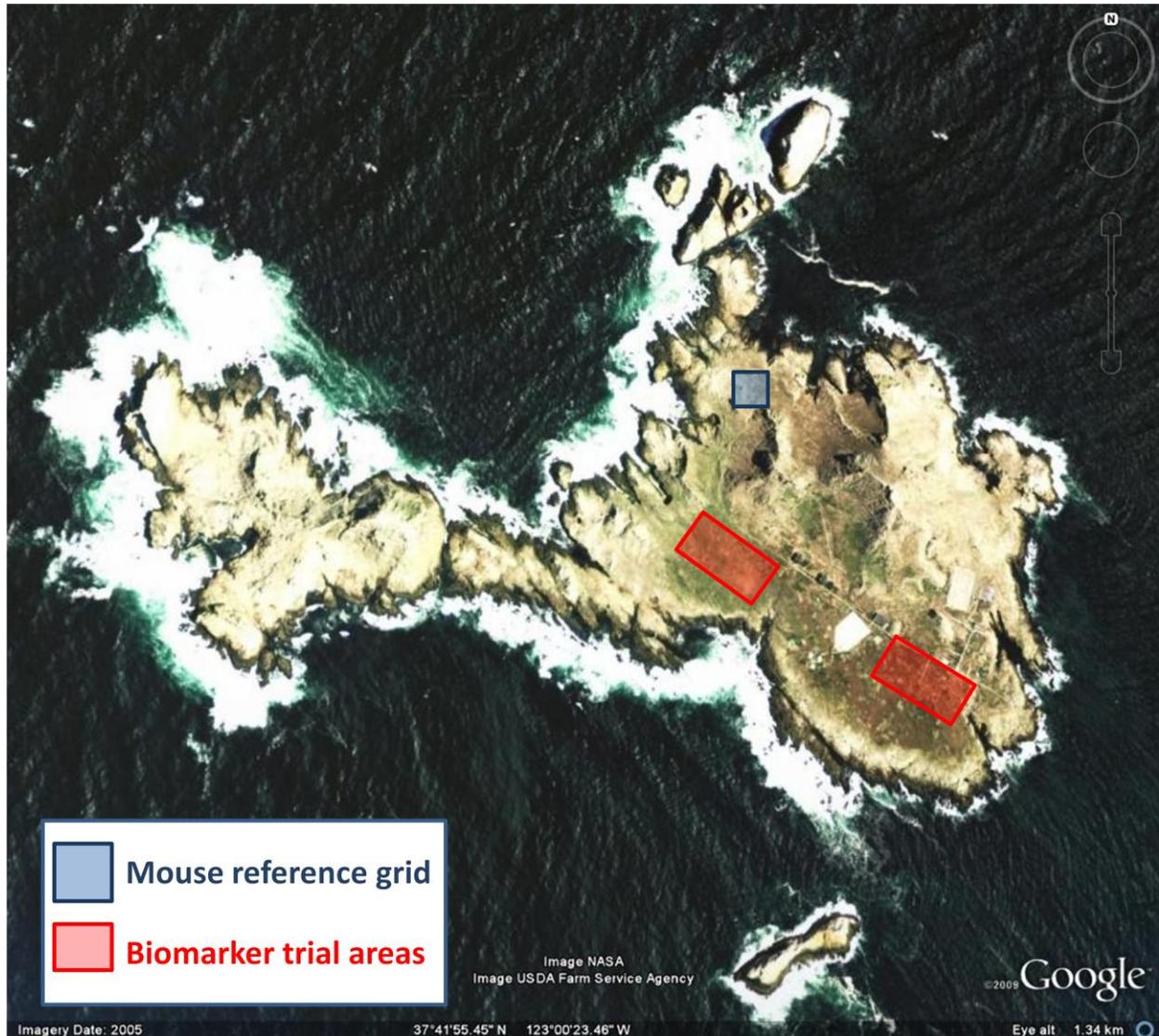


Figure 1. A map of Southeast Farallon Island showing proposed study areas for biomarker bait trial.

2.4.5 Biomarker bait detection in mice

Within the study areas mice will be trapped and screened for the presence of pyranine dye to confirm bait uptake. A 10 x 10 trap grid will be placed at the center of each study site, and positioned a distance inside the plot boundary that is twice the diameter of the mean mouse home range, as determined in the movement study (see section 2.2.2). Traps will be laid in pairs at 2-m intervals, totaling 200 traps per grid. Traps will be set in the evening five days after application, and trapped every night for five consecutive nights or for a different duration of time as indicated in the detection assay. To investigate

the effects of immigration of mice into the study area, an assessment transect will start in the core of the trapping grid and run through the baited area to at least 50 m beyond the baited area (Wanless et al. 2009). Trap stations will be placed in pairs and spaced apart by 15 m.

Once trapped, each mouse will be screened for the pyranine dye using a hand-held ultra-violet light. If a mouse shows external sign (generally indicated on anus, tail, or mouth) the animal will be euthanized and further inspected for signs of biomarker exposure in the alimentary track. Mice without external biomarker sign will be tail marked (using colored Sharpie marker) and released in the study area at their point of capture. For each trapping period the intensity of fluorescence will be recorded using the same rating scale previously described (see section 2.4.2). At the end of the study period (10th day after second bait application) mice without evidence of dye will be euthanized, collected, and frozen for further screening in a laboratory. Mouse carcasses will be buried on the island, or if facilities allow, frozen on island and removed for further analysis. Using a sub-sample of captured mice on the island, a small amount of tissue will be collected, stored in Logmire buffer, and archived for preservation of genetic information (Ross 2009).

Results will be summarized to assess proportions of mice not displaying fluorescence within the core trap grid. Additionally, proportions of mice not displaying fluorescence on assessment lines outside vs. inside the baited study area will be compared

2.4.6 Biomarker bait detection in non-target species

Compared with most circumstances on the mainland, island ecosystems are generally better candidates for aerial broadcast of rodenticide for rodent eradication because there are fewer other animal species present to consume the bait and potentially succumb to its toxic effects. On the South Farallon Islands, a bait broadcast would be timed for the winter months when there are comparatively few animals present, further minimizing “non-target” exposure to rodenticide. However, during the months of November and December, when the bait broadcast is currently proposed, many thousands of gulls may be present, as well as endemic arboreal salamanders. While none of these taxa are believed to be at risk of significant, long-term negative population impacts from an aerial rodenticide broadcast, the scale of their potential exposure to the bait on the South Farallones is not easily predictable.

In conjunction with biomarker bait trials to assess the efficacy of mouse eradication, non-target species (gulls and salamanders) will be examined as an index of the potential risk of exposure to rodenticide

during the proposed eradication. A bait application trial using non-toxic biomarker-infused bait will allow researchers to quantify exposure rates in gulls, which may consume bait pellets if they encounter them. Due to their insectivorous diet, salamanders are not likely to be exposed directly to rodenticide, but their status as an endemic subspecies isolated to the South Farallon warrants taking additional measures to examine all potential exposure pathways to rodenticide.

2.4.6.1 Western gull residents and migratory gulls

To assess the risk associated with bait application during the proposed eradication operation, resident Western and migratory gulls will be evaluated for exposure during the biomarker trial. During the proposed eradication period of Nov-Dec, large numbers of resident Western gulls return day after day to roost on their territories which are scattered widely across the islands. Migratory gulls also roost on the island in large numbers, but tend to congregate in the intertidal areas with some spillover onto upland areas. Whether migratory gulls utilize the intertidal and upland areas for foraging purposes is unknown. Following the biomarker bait broadcast, gull exposure to bait will be evaluated in the following ways:

1. Capture of a subsample of birds from each study area after bait broadcast within or immediately adjacent to study areas.
 - a. Beginning 3 days after each bait application, gulls will be trapped either individually (spotlight or snares at their roost sites) or en masse (box trap) for five consecutive days. Birds caught will be examined externally around the mouth area and cloaca for evidence of fluorescence using a UV light. The age class and presence or absence of fluorescence will be recorded for each captured bird examined. Following examination, birds will be released at their capture location. In order to avoid double-counting, captured gulls will be banded and marked with a temporary dye on the chest or head. Furthermore, the proportion of marked gulls returning to roost will be determined during the evening gull survey conducted daily by PRBO biologists.
2. Visual observation of roosting gulls from a distance using ultra-violet spotlight within and surrounding study areas.

Given the potential challenge of capturing non-breeding gulls and the larger spatial movements of gulls outside the study area, distant visual observations of roosting gulls will be made to examine for fluorescence. A UV spotlight will be used to illuminate bird

fluorescence visible around the mouth and anus area at each known roosting site on the island. Observers will be positioned ~ 50 m, or as close as possible without flushing birds, and the numbers of birds by age class displaying or not displaying fluorescence tallied. Beginning 3 days after bait application counts will be made during the evening for five consecutive days. The proportion of gulls at each roosting site displaying fluorescence will be recorded. During these surveys anecdotal observations of other bird species that could have been exposed to bait will be recorded.

3. Evaluation of gull feces in plots after bait broadcast within and at a greater spatial distance from the study areas.
 - a. Fixed plots approximately 5 x 5 m will be established within and around the study area, targeting known gull roosting sites. Prior to bait application fecal deposits within each plot will be tallied and the plot cleared of feces. Following bait application, plots will be repeatedly visited beginning 3 days after each bait application for five consecutive days. New fecal deposits within the plot will be examined for evidence of fluorescence using a UV light. At each visit the number of new deposits with or without fluorescence will be tallied, and all new deposits cleared from the plot (to reduce the likelihood of pyranine leeching during precipitation, potentially resulting in false-positive detection).

Information gathered in the above studies will be summarized as proportion of roosting birds that detected positive. The data will be used one of two ways: a simplistic model quantifying risk exposure to gulls on the Farallon Islands, based on a proportion of the roosting population exposed to biomarker. If the outcome suggests that approximately >10% may be exposed, then a more in depth risk assessment will be conducted by contracting an experienced risk assessment analyst.

Note: To carry out the above studies advice of experienced researchers on the Farallon Islands would be required, particularly those with knowledge relating to roosting and capture of gulls.

2.4.6.2 Salamanders

No native reptiles or mammals inhabit the Farallon Islands, but a population of arboreal salamanders (*Aneides lugubris*) is found across the islands. The species is endemic to California and Baja California where it is distributed primarily along the coast, with populations on some offshore islands and in the Sierra Nevada foothills.

Although not threatened, salamanders will also be evaluated for exposure during the biomarker trial. Salamanders are not expected to consume bait pellets directly, but their status as an endemic subspecies warrants further investigation. Prior to bait application temporary shelters preferred by salamanders will be installed within the study area. Beginning 3 days after each bait application and continuing for 5 consecutive days the shelters will be checked and any salamanders captured will be examined for fluorescence using a UV light. The presence/absence of biomarker fluorescence around the mouth or anus will be recorded for each captured salamander. Additionally temporary shelters will be examined for fecal material or invertebrates showing fluorescence. To avoid double-counting salamanders will be marked, using an approved method, during the time of capture.

It should be noted that results from this study may yield a false positive, as salamanders might be exposed to the dye owing to its solubility in water and the salamanders' preference for damp habitats (for example bait deteriorating in rainwater puddles and releasing the dye).

Information gathered in the above studies will be summarized as proportion of salamanders that detected positive. The data will be used one of two ways: a simplistic model quantifying risk exposure to salamanders on the Farallon Islands, based on a proportion of the population exposed to biomarker. If the outcome suggests that approximately >10% may be exposed, then a more in depth risk assessment will be conducted by contracting to an experienced risk assessment analyst.

2.5 Other variables

2.5.1 *Climate data collection*

Standard climate data will be collected during the bait application trials to account for some of the variables that might influence the results. This will include daily rainfall, minimum and maximum ambient temperatures and cloud cover.

2.6 Field schedule & resource needs

2.6.1 *Field schedule*

A proposed schedule of field activities is shown in Table 1. A total maximum time of 32 days will be required to conduct the trials as described. However, the duration of the field trial could be reduced to a

minimum time of 21 days, depending on the pyranine detection period in mice as determined in the assay trial (Section 2.4.2). Therefore, exact calendar dates of the trial are not fixed, and the termination date of the trial will be variable depending on the outcome scheduling needs or constraints on the Farallon Islands.

2.6.2 Staffing

To complete the trials as described a core group of 4 Island Conservation staff will be required. Additionally part-time support of 2-3 PRBO and/or U.S. Fish and Wildlife Service biologists would be required to assist and/or advise on aspects of the study relating to gulls and salamanders.

2.6.3 Field accommodation

Preferred accommodation for staff during the trial would be in FWS/PRBO housing, depending on availability. Alternately a tented field camp will be installed on the island to provide living and work quarters for field staff. Island Conservation will coordinate appropriately with staff at FWS/PRBO regarding field accommodations and logistics.

2.6.4 Field resource needs

Researchers will need to coordinate with FWS/PRBO staff regarding logistics, timing, and transport of field staff and equipment to the island. During field trials researchers would prefer access to shared laboratory and office space to conduct some of the studies and analysis mentioned above. Researchers would also prefer access to basic utilities on the island, including power for charging camera and laptop batteries, restrooms, and water. Alternately a temporary structure (weatherport or similar framed tent) can be installed to provide indoor working space and equipment such as a generator, water, pit toilet, etc. can be brought to the island to support the research team.

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Table 1. Proposed timeline and study activities for biomarker bait trial on the Farallon Islands.

Project activity	Nov														Dec																			
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Transit island & orient	x																																	
Establish bait uptake grid & plots		x																																
Establish mouse trap grid		x																																
Broadcast uptake study plot			x																															
Measure bait uptake				x	x	x	x	x	x	x	x	x																						
Mark-recapture mouse survey			x	x	x	x	x	x																										
Mouse detection assay			x	x	x	x	x	x	x	x	x	x	x																					
Mouse movement study				x	x	x	x	x	x	x																								
Establish mouse biomarker trap grids											x	x																						
Establish gull plots											x	x																						
Establish salamander covers											x	x																						
Establish biomarker uptake grid, plots											x	x																						
Broadcast biomarker study areas												x										x												
Measure biomarker bait uptake												x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Open biomarker traps, sample mice																x								x										
Close biomarker traps																					x									x				
Sample salamanders															x	x	x	x	x				x	x	x	x	x							
Capture gulls															x	x	x	x	x				x	x	x	x	x							
Examine gull fecal plots															x	x	x	x	x				x	x	x	x	x							
Visual gull observations																			x									x						
Demobilize study areas																																	x	
Transit off island																																	x	

Appendix 1.

Refer to attached pyranine MSDS.