

**An Assessment of the Potential Hazards of the Anticoagulant Rodenticides Diphacinone  
and Brodificoum to Salamanders**

**A Preliminary Report to the National Fish and Wildlife Foundation  
Northern Coastal California Restoration Fund**

Gary Witmer, Ph.D., Supervisory Research Wildlife Biologist  
Rodent Research Project Leader  
USDA/APHIS Wildlife Services  
National Wildlife Research Center  
4101 Laporte Avenue, Fort Collins, CO 80521-2154

And

Gerard J. McChesney  
U.S. Fish and Wildlife Service  
Farallon National Wildlife Refuge  
1 Marshlands Road  
Fremont, CA 94555

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## INTRODUCTION

House mice cause many types of damage and when introduced to islands, house mice can cause significant damage to natural resources, including both flora and fauna (Witmer and Jojola 2006). For example, on Gough Island in the South Atlantic, house mice fed on nestling albatross chicks (Cuthbert and Hilton, 2004). Additionally, Witmer et al. (2012) documented seedling damage by house mice in a pen study. House mice are omnivores, yet their diet is largely dominated by insects, some of which are likely plant pollinators (Shiels et al., 2013; Shiels and Pitt, 2014). House mice are subordinate to introduced rats so the impacts of mice may go unnoticed when rats are also present on the island (Angel et al., 2009). This phenomenon was demonstrated by the large increase in mice abundance on Buck Island, U.S. Virgin Islands, after invasive roof rats were eradicated (Witmer et al., 2007a). In very dry habitats on islands, house mice may numerically dominate over introduced rats.

There have been numerous successful eradications of invasive rodents on islands (Howald et al. 2007, Witmer et al. 2011) and these projects have relied upon rodenticides for their completion (Witmer et al. 2007b). APHIS maintains the registrations for two rodenticide active ingredients for invasive rodent eradication: diphacinone and brodifacoum. However, rodenticides can pose hazards to non-target wildlife so careful considerations and measures must be taken to reduce those risks (Witmer et al. 2007b).

Invasive house mice are present on the Farallon National Wildlife Refuge (NWR) and are causing damage to seabirds, the endemic Farallon arboreal salamander (*Aneides lugubris farallonensis*), terrestrial invertebrates, native plants, and may be dispersing weed seeds (USFWS 2013). Hence, the U.S. Fish and Wildlife Service (USFWS) has proposed a plan to eradicate the invasive mice from the refuge (USFWS 2013). Proposed action alternatives to eradicate the mice include broadcast of either diphacinone or brodifacoum. As part of the planning process, the USFWS would like an assessment of the potential hazards of these anticoagulants to salamanders. USFWS is collaborating with the USDA/APHIS/WS's National Wildlife Research Center (NWRC) to conduct the assessment based on the extensive animal research facilities and staff as well as previous experience of assessing hazards of anticoagulants to reptiles (Witmer and Mauldin 2012).

The objective of this study is to assess the potential hazards of the rodenticides diphacinone and brodifacoum to salamanders. We will expose the salamanders to the rodenticides through two routes: 1) secondary oral exposure by allowing the salamanders to consume insects that have fed upon anticoagulant pellets, and 2) direct external exposure by allowing salamanders to be exposed to crushed pellets and water that has been used to soak anticoagulant pellets thus allowing dermal absorption. We hypothesize that the rodenticide exposure will cause some mortality or other sub-lethal effects (decline in food consumption and/or loss of weight). Since our proposal to NFWF, this study has been expanded with other funds to evaluate exposed salamanders for anticoagulant residues and for testing salamanders for exposure to live-threatening pathogens. We will necropsy salamanders that die during the trial or after they are euthanized at the end of the study for signs of internal bleeding (Stone et al. 1999). We will also test the salamander carcasses for anticoagulant residues at the end of the study.

## PROGRESS TO DATE

Due to the expansion of the study and other factors, this study has not yet been completed. In addition to funds provided by the NFWF Coastal Restoration Fund, funds were obtained from the NFWF North Pacific Seabirds initiative and from the DOI Restoration Catalyst Fund. These additional funds have allowed us to expand the study beyond the initial scope, increasing the time needed to complete the study. Here, we provide a summary of progress to date. A more extensive, final report will be provided at the completion of the entire project.

Several conference calls were held between USFWS, Department of Interior's Restoration Support Unit (DOI), NWRC, San Francisco State University (SFSU) and other experts. Discussions centered around the need for the study, potential approaches, which species of salamanders to use, the numbers of animals needed, the rodenticide exposure routes, and schedules. Then a work plan and budget were completed along with the interagency agreement between FWS and NWRC. From those discussions, it was decided that two study species for the trials would be used in the study, *Aneides lugubris* and the closely related *Ensatina eschscholzii xanthoptica*. Study organisms would be live-captured from wild stock. *Ensatina* would provide the majority of the sample size because of their much greater abundance in the wild, while a smaller sample of *Aneides* (the same species present on the Farallon Islands) would be examined for confirmation of results shown in *Ensatina*. Salamanders would be captured on the mainland of the San Francisco Bay area.

A scientific literature review was completed to assist with drafting the study protocol and making decisions on approaches, procedures, and salamander maintenance. We also searched for research articles that had assessed the hazards of various chemicals to amphibians. While we located articles on pesticides such as herbicides and insecticides and on metals and acidic water, we found no articles on anticoagulant rodenticide hazards.

A required consultation with the NWRC attending veterinarian was held to discuss the proposed study. A SFSU-approved SOP on salamander maintenance was obtained from that institution and incorporated into the study protocol. The draft study protocol was reviewed by the NWRC's Institutional Animal Care and Use Committee (IACUC) and NWRC's Quality Assurance Unit, revised, and formally approved by those groups and the NWRC Director's Office.

The study director, Dr. Gary Witmer, met with the animal care staff (AC) to give an overview of the study and to discuss the maintenance procedures for the salamanders. He also consulted with the leader of the NWRC's Analytical Chemistry Unit to discuss the rodenticide residue analyses that would be needed at the end of the study.

Two tropical, animal research rooms were reserved and the room computers were programmed to the desired temperature, humidity, and light cycle. There was one room for each of the two species of salamanders we decided to use in the study. Various supplies for the study were purchase, including terrariums and lids, unbleached paper towels, and the anticoagulant rodenticides. Sixty terrariums were set up with 30 in each animal research room. We searched websites for sources of crickets of various sizes for feeding the salamanders and purchased needed supplies. Data collection sheets were also designed and printed off for each room.

The USFWS contracted with SFSU under a separate hand-capture wild salamanders for the study. The protocol called for testing captured salamanders for pathogens and quarantining salamanders for health examinations. Salamanders passing health exams were then shipped overnight to NWRC in Fort Collins, Colorado. In order to do this, they needed to have their salamander scientific collecting permit modified and approved. A delay in this process caused further delays in providing study animals to NWRC.

At this time, this study is on-going. The NFWF NCCRF portion of the funds have been expended. A more thorough final report, with all results, will be provided at the completion of the study. The study is expected to be completed in late March 2017.

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