

**Preliminary Report, QA-2688:**

**An assessment of the potential hazards of anticoagulant rodenticides to salamanders**

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## BACKGROUND

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 animals so careful considerations and measures must be taken to reduce those risks (Witmer et al. 2007b).

Invasive house mice are present on the Farallon Islands National Wildlife Refuge (NWR) and are causing damage to seabirds, the endemic arboreal salamander (*Aneides lugubris farallonensis*), terrestrial invertebrates, native plants, and may be dispersing weed seeds (Farallon National Wildlife Refuge 2006, Island Conservation Undated). Hence, the US Fish and Wildlife Service (FWS) would like to eradicate the invasive mice from the refuge (Farallon National Wildlife Refuge 2006, Island Conservation Undated). For inclusion in their EIS document, the USFWS would like an assessment of the potential hazards of anticoagulants to salamanders. They have requested that the USDA/APHIS/WS's National Wildlife Research Center (NWRC) conduct the assessment based on our extensive animal research facilities and staff and our 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 brodifacoum and diphacinone 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). 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

Several conference calls were held between FWS, Department of Interior's Restoration Support Unit (DOI), NWRC, and San Francisco State University (SFSU). 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.

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 (*Aneides lugubris* and the closely related *Ensatina eschscholzii xanthoptica*). 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. Data collection sheets were also designed and printed off for each room.

The FWS contracted SFSU under a separate agreement to hand-capture the salamanders from the mainland of California for the study. They are to hold the salamanders until they have enough to ship to NWRC in Fort Collins, Colorado. In order to do this, they needed to have their salamander scientific collecting permit modified and approved. They also agreed to test the salamanders at the university for the chytrid pathogen that has been decimating amphibian populations. They will not ship any salamanders infected with the fungus. Unfortunately, the extensive drought in California has delayed the collecting of the salamanders because there is very little activity under very dry conditions. However, recent heavy rains should allow the collecting to begin soon.

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