Migration of Brodifacoum and Diphacinone from Bait Pellets into Topsoil at Palmyra Atoll National Wildlife Refuge

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Abstract: Between June 1 and 30, 2011, a partnership between the U.S. Fish and Wildlife Service, The Nature Conservancy, and Island Conservation successfully implemented a project to remove introduced black rats from Palmyra Atoll National Wildlife Refuge. Prior to the rat eradication, we assessed several environmental risk factors associated with the application of an anticoagulant rodenticide to Palmyra's emergent land area. Here, we present the findings from a study of toxicant migration from bait pellets into topsoil. Topsoil from plots characterized as "sandy" or "humus" was collected after exposure to bait pellets containing 50 ppm of diphacinone or 25 ppm of brodifacoum. Brodifacoum and diphacinone were detected in samples of both sandy and organic topsoil while control samples collected outside of the study plots tested negative for both toxicants. With both toxicants, residue concentrations decreased with time and neither toxicant was detected in most of the 28, 36, and 50-day samples; trace amounts (≤0.2 ppm for brodifacoum and ≤2 ppm for diphacinone) of the toxicants were detected in a few samples from these groupings. We did not find a significant difference in toxicant concentrations between the two types of topsoil. The results from this study suggest that following a broadcast of rodenticide across Palmyra's emergent land area, small amounts of brodifacoum or diphacinone would migrate to, and remain in, Palmyra's topsoil for a short period of time.

Key Words: anticoagulants, black rat, brodifacoum, diphacinone, environmental fate, non-target species, Palmyra Atoll, *Rattus rattus*, residues, rodenticides

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INTRODUCTION

Toxicants deployed during campaigns to control or eradicate invasive rodents may persist in the environment, creating potential exposure pathways for non-target organisms. Anticoagulants are the most widely used rodenticides worldwide (Eason et al. 2002, Hoare and Hare 2006); however, the migration of toxicants from the bait matrix into soil, and the persistence of toxicant in soil mediums, has rarely been studied in laboratory (Newby and White 1978, Jackson et al. 1991, Hall and Priestly 1992, Jackson and Ashton 1992), or in natural settings (Ogilvie et al. 1997, Orazio et al. 2009, Fisher et al. 2011).

In 2011, the U.S. Fish and Wildlife Service (USFWS) produced an Environmental Impact Study for the proposed eradication of introduced black rats (*Rattus rattus*) from Palmyra Atoll (USFWS 2011). All action alternatives considered in the EIS involved the deployment of rat bait containing an anticoagulant toxicant to Palmyra's emergent land area. To inform the alternative selection process, the USFWS commissioned Island Conservation to assess the migration of two rodenticides, diphacinone (1st-generation anticoagulant) and brodifacoum (2nd-generation anticoagulant), from two bait products to the topsoil, and to assess the short-term persistence of each compound in Palmyra Atoll's substrate.

Both compounds have a high affinity for organic matter (U.S. EPA 1998), low solubility in water, and strong soil adsorption properties (World Health Organization 1995). Neither toxicant is mobile in water, air, or soil (Eason and Wickstrom 2001). The half-life of brodifacoum in soil under aerobic conditions is reported to be 157 days, and about 30 days for diphacinone (World Health Organization 1995, Haydock and Eason 1997, U. S. EPA 1998). Persistence in a specific environment will depend on several factors such as soil type, sunlight, temperature, microbial activity, and nutrient concentrations (World Health Organization 1995, Kamrin 1997).

We report the results of residue analyses undertaken on soil exposed to brodifacoum and diphacinone under natural field conditions. The analyses were undertaken to investigate the migration of brodifacoum and diphacinone into topsoil following a broadcast rodenticide application to Palmyra's emergent land areas.

METHODS

Palmyra Atoll National Wildlife Refuge is a tropical atoll located at 6° N and 162° W in the Line Islands of the Central Pacific Ocean. Located within the low pressure area of the Intertropical Convergence Zone, the atoll has a hot, extremely wet equatorial climate with over 4,000 mm of rainfall each year. Rainfall events produced extremely wet conditions across the atoll, with 1,477 mm recorded during the study period. Palmyra consists of 250 ha of emergent land comprised of two predominant soil types. Within forests of *Pisonia grandis* (Lost Island), soils are highly phosphatic and composed almost entirely of organic matter (Christophersen 1927); this soil type is hereafter referred to as "humus." Soils associated with

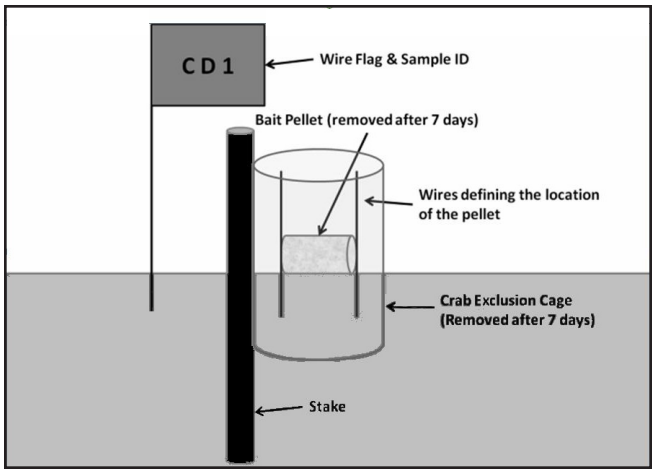

Figure 1. Components of the pellet cage.

Cocos nucifera (coconut palm) forests (Cooper Island) are sandy and non-phosphatic with medium or low organic matter (Christophersen 1927); this soil type is referred to as "sandy."

Two types of cereal-based pellets were tested: one contained 25 ppm brodifacoum (Brodifacoum 25W Conservation, EPA Reg. No. 56228-37, Bell Laboratories, Inc., Madison, WI), hereafter referred to as "25W", and the other contained 50 ppm diphacinone (Diphacinone 50 Conservation, EPA Reg. No. 56228-35, Hacco, Inc., Madison, WI), hereafter referred to as "D50". Pellets were placed in direct contact with soil to mimic the migration of the anticoagulant compounds into Palmyra's topsoil after bait application. 25W is specifically for use in wet conditions and is designed to break down slowly after exposure to moisture in humid rainy environments.

From April 8 to May 28, 2010, the environmental fate of the matrix and active ingredients of 25W and D50 were evaluated. The pellets were placed inside exclusion cages that consisted of a 7.6 × 5.1-cm section of PVC with 0.6-cm wire mesh affixed to the top (Figure 1). The cages prevented rats and land crabs from removing or disturbing pellets, and they allowed the pellets to be exposed to environmental factors, such as rain and humidity, and small invertebrates. One plot was established per location and per exposure period. Each plot included 3 cages with diphacinone placed in the same microhabitat (1 m^2), and separated from 3 cages containing brodifacoum by 3-5 m. Topsoil to 2 cm in depth was collected directly beneath pellets after 2, 7, 28, 36, and 50 days of exposure, and pellet condition was observed. Reference samples of topsoil were collected at least 5 m away from each study plot.

To collect topsoil samples, we drove a stainless steel tube (2.5 cm inside diameter × 70 cm length) to a vertical depth of 8 cm beneath the spot where each pellet had sat and then extracted the tube and collected the soil core on a clean surface; bait pellets, if still intact, were removed prior to the collection of the soil samples. The stainless-steel tube was washed with acetone and fresh water to remove particulate matter and toxicant residue before collecting each sample. Approximately 0.5 - 1.0 g of topsoil was collected from a section of the core that was 8 ± 2 cm in depth. The samples were transferred into sterile plastic vials with secure lids and then stored in a -80°C freezer until they were transported to the California Animal Health and Food Safety Laboratory at the University of California, Davis for analysis (Laboratory SOP: CAHFS DTOX-02-805) (Palazoglu et al. 1998).

Anticoagulant Analysis and Data Analysis

Soil samples were well mixed, and 0.25-gram of sample was extracted with 2.5 mL of methanol by sonication and rotation of the test tube for 1 hour. After centrifugation at 2,000 rpm for 5 minutes, the extracts were cleaned up by filtration though a 0.45 µm syringe filter (Waters Millex®-HV, 0.45 µm) into autosampler vials.

To increase diphacinone sensitivity, a 0.2 ml aliquot of the sample extract was concentrated two-fold by blowing the extract to dryness under a nitrogen stream and re-constituting it in 0.1 ml of methanol. Analysis for diphacinone was done by high-pressure liquid chromatography with diode array detection at 325 nm. The injection volume was 10 µl. Soil and sand samples were used as control matrices and fortified with diphacinone at 2 µg/g. The limit of quantitation for diphacinone in the soil samples was 2 µg/g. The limit of quantitation for diphacinone was higher than that for brodifacoum because diode array detection is less sensitive than fluorescence detection. However, diphacinone does not fluoresce, and thus it has to be analyzed by diode array detection.

Analysis for brodifacoum was done by high-pressure liquid chromatography (HPLC) with fluorescence detection using 390 nm emission and 310 nm excitation. The injection volume was 10 µl. Both soil and sand samples were used as control matrices and fortified with brodifacoum at 0.2 µg/g. The limit of quantitation for brodifacoum in the soil samples was 0.2 µg/g. The analysis allowed for detection of brodifacoum below the limit of quantitation, which was indicated as a "trace" amount. A value of half of the reporting limit was assigned to trace results for statistical analysis.

Data were accepted when the results of the HPLC analysis matched the analyte's spectrum and retention time of that of the certified standard reference material, and when recovery of the analyte in the associated fortified sample was between 75% and 110%. In addition, negative controls showed no presence of brodifacoum or diphacinone in the respective analyte region.

A one-way analysis of variance (ANOVA) was conducted to compare the concentration of brodifacoum and diphacinone in each of the soil types over four independent exposure periods, reported with degrees of freedom (DF), F-statistic (F), and the p-value. Statistical analysis was conducted in Minitab® 16 with an alpha level of 0.05 for all tests.

RESULTS

Low concentrations of both rodenticides were detected in 20 samples of sandy and humus topsoil. Of 54 samples (Table 1), only two contained rodenticide concentrations that were above the reporting limits. The rest of the samples yielded a zero value (the toxicant was not detected) or a 'trace' value. Neither rodenticide was detected in the control samples.

With the samples of humus topsoil, we found no sig-

Table 1. Number of samples per location per day collected from two soil types containing each toxicant. (-) indicates no samples were taken at that location. The number of samples with quantified residue concentrations, the number with trace detections, and the number of total samples are listed respectively (positive / trace / total collected).

Sample Period	Cooper Island (Sandy Soil)		Lost Island (Organic Soil)		Control
	25 W	D50	25W	D50	
2 days	1/2/3[1]	0/0/3	0/2/3	0/2/3	0/0/2
7 days	1/2/3[2]	0/0/3	0/3/3	0/2/3	0/0/2
28 days	0/1/3	0/0/3	0/2/3	0/0/3	0/0/1
36 days	0/0/3	0/0/3	-	-	-
50 days	-	-	0/2/3	0/0/3	0/0/1

[1] Day 2 25W sandy sample reported at 0.2 ppm

[2] Day 7 25W sandy sample reported at 0.8 ppm

Table 2. Description of the condition of bait pellets subject to different exposure periods. Each plot represents an independent sampling period, and the plots, as a group, do not represent a time sequence. For each rodenticide, 3 pellets were observed per plot, per exposure period (n= 48 pellets). Bait pellets that were not intact but had crumbs or other visible evidence remaining were classified as disintegrated. (-) indicates non-applicable at that location.

Exposure Period for Independent Plots	Bait Pellet Condition			
	Cooper Island (Sandy Soil)		Lost Island (Organic Soil)	
	25 W	D50	25W	D50
2 days	Intact	Disintegrated	Intact	Disintegrated
7 days	Gone	Gone	Intact	Gone
28 days	Gone	Gone	Intact	Disintegrated
36 days	Gone	Gone	-	-
50 days	-	-	Gone	Gone

nificant difference in the concentration of brodifacoum (DF= 3, F= 0.33, P=0.80) or diphacinone (DF= 3, F= 2.67, P= 0.11) between exposure periods. Similarly, we did not detect a difference in brodifacoum concentrations in samples of sandy soil between the exposure periods (DF=3, F=1.59, P= 0.26); diphacinone was not detected in any of the sandy soil samples, although trace detections were found in humus soil on day 2 and day 7 (Table 1).

Bait softened due to uptake of moisture from Palmyra's humid environment, and it eventually disintegrated into crumbs or fragments. Bait condition was qualitatively assessed on the last day of each exposure period and characterized by the form most frequently observed (Table 2). Bait condition was consistent among local replicates, but differed among study sites. Bait on Cooper Island (sandy soil) disappeared quickly, though bait was frequently found intact or disintegrated on Lost Island. In general, brodifacoum bait persisted longer than diphacinone bait. Brodifacoum pellets were observed intact on organic soil despite 7 days of exposure, in contrast to diphacinone pellets that disintegrated after two days. Despite moisture resistant properties, brodifacoum pellets at the sandy soil site disappeared between day 2 and day 7, likely due to consumption by small invertebrates or other methods of degradation, while in the humus soil area brodifacoum pellets remained mostly intact for 7 days.

DISCUSSION

Topsoil that was in contact with bait pellets for 2 to 7 days contained low (less than 1 ppm) or undetectable concentrations of brodifacoum or diphacinone. The dearth of detectible concentrations of brodifacoum or diphacinone in the soil samples suggests that the migration of rodenticide from the tested bait products to the topsoil upon which the pellets sat is minimal, regardless of exposure time. Significant soil contamination is unlikely after a broadcast application of bait. Toxicant residue from brodifacoum and diphacinone bait products does not appear to be readily incorporated or retained in either of Palmyra's two primary soil types. The results of this current study are in agreement with other findings (Morgan and Wright 1996, Ogilvie et al. 1997, Orazio et al. 2009) that suggest rodenticides used in broadcast eradications do not transfer high concentrations of toxicants to soil, even in warm, moist conditions where the degradation of bait matrices is rapid. The active ingredients in rodenticides are known for the potential to persist in the environment (Primus et al. 2005, Sage et al. 2007, Fisher et al. 2011), but the low concentration used in bait pellets limits their potential for significant environmental contamination.

A bait availability study on Palmyra Atoll indicated that an application rate of 85-95 kg/ha would be adequate to overcome competition from the high abundance of land crabs and hermit crabs that consume bait (Buckelew et al. 2005). A brodifacoum broadcast on Palmyra Atoll using an application rate of 85 kg/ha would require 21,250 kg total bait, resulting in 0.532 kg (0.0025%) of active ingredient. Even with abundant daily rainfall, our results suggest that only a small amount (0.8 ppm of the original 25 ppm, or approximately 3%) of the original toxicant is detected in soil up to 7 days after the application, and the rest is either consumed or degraded at the surface. Even if bait pellets remained in contact with soil for 7 days post-application, only trace concentrations of brodifacoum and diphacinone residue would remain afterwards. The minimal toxicant integration into Palmyra's soil reduces the risk of significant long-term environmental contamination.

The difference between degradation rates for the two study sites could be due to spatial differences in invertebrate (ant) or microbe populations, though this was not measured. Nutrient availability differs among Palmyra's soil types. Concentrations of $\delta^{15}N$, NH_4^+, NO_3 are significantly higher in dicot (*Pisonia grandis*) patches than in monocot (*Cocos nucifera*) patches (Young et al. 2010). The availability of nitrogen in soil influences microbial

community composition, biomass, and respiration (Soderstrom et al. 1983, Waldrop et al. 2004, Treseder 2008, Janssens 2010); therefore, soil type could be a determining factor in the persistence of rodenticides at Palmyra Atoll. We did not find a significant difference between rodenticide concentrations in samples of topsoil and soil type; however, this could be a result of the relatively short study period and the detection limits. Due to the difference in sensitivity of the instrumentation for diphacinone and brodifacoum, there is a difference in reporting limits. At the time of analysis, it was not possible to lower the reporting limit for diphacinone.

Degradation of the pellet matrices may have been expedited by greater than usual rainfall (1,477 mm) at Palmyra Atoll during the sampling period. Breakdown of bait starts as soon as rain begins to fall following bait application (Haydock and Eason 1997). Brodifacoum 25W is manufactured with sorbitol, a sugar alcohol that prevents pellet fragmentation in moist environments; therefore, 25W pellets retained shape and cohesiveness longer than did D50 pellets.

The potential for long-term contamination of soil following a rodenticide broadcast on Palmyra Atoll is extremely low for products containing both brodifacoum and diphacinone. Caution should be used in extrapolating general lack of mobility and persistence of toxicants at Palmyra Atoll to other environments. Evidence of toxicant persistence beyond the levels detected in this study has been observed. In one instance, residual concentrations of brodifacoum were detected in soil samples from forest and grassland sites following an aerial broadcast application off New Zealand. Detection levels decreased to near the limit of detection after 100 days (Fisher et al. 2011). Information gaps exist around the physical and biological factors that enable toxicants like brodifacoum and diphacinone to persist under various environmental conditions.

This study provided insight into one of many aspects of rodenticide-based pest management projects, yet further study is required to fully understand the associated risk to non-target wildlife. As more information about the migration of toxicants through a variety of soil types and ecosystems becomes available, project planning can be refined to ensure that non-target impacts from eradication campaigns are minimal. Through careful and proper application of rodenticides, non-target populations and other island resources will recover from eradication activities rapidly, and continue to benefit over time once negative impacts that introduced species often bring to island flora and fauna are eliminated.

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