

**USDA APHIS Wildlife Services  
National Wildlife Research Center  
Hawaii Field Station**

**Mongoose Control Method Development FY19 Final Report:**

**Efficacy trial of a mongoose toxicant for use as a conservation tool in Hawai'i**

Shane R. Siers<sup>1</sup>, Robert T. Sugihara<sup>1</sup>, Emily W. Ruell<sup>2</sup>, Israel L. Leinbach<sup>1</sup>,  
Daniel Sedgwick<sup>1</sup>, Thomas W. McAuliffe<sup>1</sup>, Are Berentsen<sup>3</sup>, Chris N. Niebuhr<sup>1,4</sup>, and  
Steven C. Hess<sup>1</sup>

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<sup>1</sup> USDA APHIS Wildlife Services, National Wildlife Research Center, Hawaii Field Station, Hilo, HI

<sup>2</sup> USDA APHIS Wildlife Services, National Wildlife Research Center, Registration Unit, Fort Collins, CO

<sup>3</sup> USDA APHIS Wildlife Services, National Wildlife Research Center, Rabies Project, Fort Collins, CO

<sup>4</sup> Current affiliation: Manaaki Whenua – Landcare Research, New Zealand, niebuhr@landcareresearch.co.nz

## EXECUTIVE SUMMARY

With the support of Hawaii Invasive Species Council (HISC) FY2018 funding, the Hawaii Field Station and Registration Unit of the Wildlife Services National Wildlife Research Center (WS-NWRC) performed cage trials evaluating the acceptance by mongoose of four nontoxic bait matrices (NWRC Study Protocol QA-2832, final report attached as Appendix A<sup>5</sup>). The results of this study concluded that all four test materials could prove suitable as potential matrices for a toxic mongoose bait, and that the final candidate would be selected based on usability, durability, compatibility with the selected toxicant, and availability from a commercial pesticide manufacturer.

HISC FY2018 funding also supported a NWRC Registration Unit/Hawaii Field Station review of the registration and use prospects for potential toxicants to be paired with a selected bait matrix, included in this report as Appendix B<sup>6</sup>. Considering the decision factors included in this review, and determining the fastest and most cost-effective path to making a mongoose bait available to conservation practitioners, we concluded that diphacinone was the best candidate toxicant given known efficacy in mongooses, previous registration of products containing diphacinone for mongoose control in Hawaii, and familiarity of practitioners and pesticide regulators with the chemical.

Although the NWRC-produced pork loaf product<sup>7</sup> had the highest consumption rates, it was not selected as most desirable matrix due to uncertainty about durability (e.g., tendency to spoil) and lack of a commercial manufacturer. The matrix with the second-highest consumption was a nontoxic version of “FOXSHIELD® Fox Bait” (Animal Control Technologies Australia (ACTA); Somerton, Victoria, Australia). FOXSHIELD Fox Bait is a fish-based pesticide bait formulated containing Compound 1080 (sodium fluoroacetate) that is commercially available for control of introduced foxes in Australia.

HISC FY2019 funding supported the necessary work to establish a testing and registration pathway to coupling diphacinone with the nontoxic fish-based FOXSHIELD bait matrix as a potential solution for a toxic bait for mongoose control in conservation areas in Hawaii and other islands with invasive mongooses globally. Custom baits can be formed in pressed blocks or sectioned sausage-skin wrapped cylinders.

We were able to establish ACTA as a partner to produce a version of their fish-based bait with diphacinone, both in small batches for research and development testing purposes and potentially as the future commercial manufacturer of a registered product for mongoose control in the U.S. We also secured the cooperation of Bell Laboratories (Madison, WI, USA) to provide their technical diphacinone, a registered manufacturing use pesticide, for ACTA to incorporate into their fish-based bait matrix.

<sup>5</sup> This final report includes results that were not included in the FY18 Final Report to HISC.

<sup>6</sup> A draft of this publication was included in the FY18 Final Report to HISC; the attached paper is the final published version.

<sup>7</sup> Originally developed as a bait for invasive Brown Treesnakes in Guam

As per the objectives of the HISC FY2019 funding agreement, we are in the process of preparing for cage efficacy trials of a fish-based bait for mongooses (0.005% diphacinone). This trial will be conducted to Good Laboratory Practices (GLP) standards, for eventual submission to the United States Environmental Protection Agency (EPA) in support of a pesticide registration. Additional data, including field efficacy trials, will likely be required before EPA approves registration for use in conservation areas.

The remainder of the body of this report is background on the need for invasive mongoose control tools and prior research on toxic baits for mongoose control (Part I) and the summarized study protocol submitted to EPA for their concurrence on the suitability of the study design (Part II). Delays associated with the COVID-19 outbreak and public health response, in addition to the required EPA protocol review period, have pushed the timeline for this study out to approximately August 2020. HISC funds were used to support the study up through April of 2020, and the remainder of the expenses will be funded from the NWRC Hawaii Field Station budget. A breakdown of expenditures of HISC funding is included as Part III.

## **PART I: BACKGROUND<sup>8</sup>**

Small Indian mongooses (*Herpestes auro punctatus*), introduced to Hawaii, Puerto Rico, the U.S. Virgin Islands, and numerous other sites worldwide, are serious predators of native wetland, seabird and upland forest avian species (Nellis and Everard 1983; Yamada and Sugimura 2004; Hays and Conant 2007). Mongooses are well established across most of the main Hawaiian Islands (Hawaii, Oahu, Maui and Molokai) where they pose a threat to the eggs and nestlings of native ground-nesting birds (Hays and Conant 2007). The threat of accidental or intentional introductions to other mongoose-free islands in the Hawaiian chain (e.g. Kauai, Lanai) and other Pacific locations highlights the need for a comprehensive array of control techniques, including attractive and palatable baits and effective toxicants, to quickly respond to reported sightings or incipient mongoose populations (Pitt et al. 2015; Phillips and Lucey 2016; Berentsen et al. 2018). Mongooses also present a health risk to humans as hosts of leptospirosis in Hawaii (Wong et al. 2012) and the Caribbean (Everard 1976), and as a rabies reservoir on several islands in the Caribbean (Seetahal et al. 2018).

Various strategies have been used to reduce or remove mongoose populations in Hawaii and elsewhere, including trapping and toxic baits. Trapping has been useful in reducing mongoose populations and predation in and around targeted sensitive native areas (ground-nesting upland and seabird colonies). Trapping, however, is labor-intensive, expensive, and only removes mongooses from a limited area (Barun et al. 2011, Sugihara et al. 2018, Berentsen et al. 2018). Toxic baits can provide a more effective and longer-lasting approach to eradicate mongooses from a larger area.

Earlier studies by Keith et al. (1989) found diphacinone to be highly toxic to mongooses with a lethal dose (LD50) of 0.18mg/kg body weight. Successful lab and field efficacy trials with

<sup>8</sup> This section was primarily written by Robert Sugihara, with review and edits by the other co-authors of this report

diphacinone formulated in a fresh meat bait culminated in a local registration (SLN Reg. No. HI-91004, EPA Reg. No. 12455-9). The SLN label allowed registered applicators to formulate 0.00025% (2.5ppm) of diphacinone in fresh ground beef placed in tamper-proof bait stations deployed in the field to protect ground-nesting native birds. At the registered concentration (0.00025%) the fresh bait had to be maintained in bait stations over an extended period (up to 14 days) to cause mortality by multiple days of feedings by mongooses. The logistics of applicators having to prepare fresh bait formulations regularly, limited bait longevity and other constraints resulted in discontinuance of the SLN registration, mainly due to limited use (Sugihara et al. 2018).

Two commercial diphacinone rodenticide bait products were subsequently approved for mongooses. These rodenticide baits, co-labeled for rats and mongooses, were formulated at 0.005% (50ppm) active diphacinone, the active concentration of most diphacinone baits registered for rats and mice. “Eaton’s® All Weather Bait Blocks Rodenticide with Fish Flavorizer™” (0.005% diphacinone, SLN Reg. No. HI-97-007, EPA Reg. No. 56-44) and “Ramik® Mini Bars Kills Rats and Mice” (0.005% diphacinone, SLN Reg. No. HI-98005, EPA Reg. No. 61282-26) are both hard, waxy, grain-based, bait blocks used in bait stations to control rats and mice. The Eaton’s product was eventually discontinued in 2004 due to rapid deterioration in the hot and humid environment in Hawaii and concerns of viable exotic plant seeds in the bait matrix (R. Sugihara, pers. comm.). The efficacy of the Eaton’s product was variable in limited field data, suggest that this bait was less successful in areas with low mongoose density or high alternative prey density (Smith et al. 2010).

Recent WS-NWRC cage feeding trials (QA-2196; Sugihara et al. 2018) of several commercial rodenticide baits indicated that the inefficacy of commercial rodenticide formulations to mongooses was likely due to the hard consistency of grain-based pellets and blocks which are not appropriate to the dentition and feeding modes of mongooses. The registered Ramik product had a fairly low efficacy (20% mortality) over a 5-day feeding period in a laboratory no-choice efficacy trial, which was likely due to low palatability and consumption of the bait rather than low toxicity to mongooses (Sugihara et al. 2018). The Ramik product remains the only registered toxicant bait available for mongoose control in the U.S., and this registration is state limited to Hawaii.

As part of the QA-2196 trials (Sugihara et al. 2018), technical diphacinone along with other candidate toxicants was formulated in fresh raw chicken, a more attractive bait matrix than the hard rodenticide bait blocks and offered to mongooses in similar 5-day feeding trials. At a concentration of 0.005% (50ppm), the normal dosage of commercial diphacinone-based rodenticide baits, technical diphacinone formulated in raw minced-chicken was found to be highly palatable to mongooses with 100% daily consumption of the fresh bait offered. The overall mortality rate was 70% for mongooses after a single day of feeding and 100% for mongooses over a 3-day feeding period. In cooperation with Japanese researchers attempting to control mongooses on Okinawa and Amami-Oshima, Japan, the 50ppm diphacinone minced chicken bait was found to be equally efficacious for mongooses in lab cage and field enclosure trials conducted in Okinawa (R Sugihara, 2016 and 2018 Japan trip reports). Subsequent

experimental field trials with the diphacinone-minced chicken bait was conducted on Amami-Oshima in isolated locations along steep terrain where trapping was not feasible. Preliminary results show that the diphacinone bait was successful in eliminating the remnant mongoose population from the baited areas (T Jogahara, University of Okinawa, pers. comm.). This demonstrates the potential for optimizing the susceptibility of diphacinone to mongoose in another more palatable bait matrix with a reduced bait exposure period (Sugihara et al. 2018).

Development of an effective mongoose diphacinone bait will require a softer, palatable, more durable bait matrix that is longer lasting in the field than fresh raw meat. A recently completed lab study (QA-2832; Siers et al. 2020, Appendix A) evaluated the palatability of four candidate non-toxic bait matrices for mongooses to determine which had adequate palatability (are consumed in sufficient amounts) to warrant future consideration as a diphacinone bait matrix. The selected candidate bait matrix was the non-toxic version of the fish-based FOXSHIELD Fox Bait. The non-toxic FOXSHIELD bait matrix was easy to handle and readily consumed by mongooses in the cage feeding trials (Siers et al. 2020).

Additionally, a toxicant registration evaluation was recently conducted for mongooses in Hawaii by WS-NWRC (Ruell et al. 2018). Of the four toxicants evaluated, a diphacinone bait for mongooses would likely be the least expensive and fastest candidate to be reviewed and approved for mongoose control by the regulatory agencies, largely due to the abundance of registered diphacinone products and the supporting registration data already available for diphacinone.

The Environmental Protection Agency (EPA) requires laboratory efficacy data for vertebrate pesticide products in accordance with EPA OPPTS 810.1000 guidelines to support the issuance of a future Experimental Use Permit (EUP) for a larger field efficacy study and a subsequent full registration application. Building on the promising results from these previous studies, this proposed two-choice laboratory efficacy study of a bait consisting of the fish-based bait matrix containing 0.005% diphacinone continues the momentum toward the eventual goal of field deployment of an effective toxic bait for mongoose control in agriculture, biosecurity, and conservation applications.

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## **PART II: STUDY OUTLINE**

### **Two-choice laboratory efficacy test in mongooses – fish-based bait for mongoose (0.005% diphacinone)**

*This protocol outline, which is currently under review and subject to EPA concurrence, was primarily drafted by Robert Sugihara and Emily Ruell with input from the other co-authors on this report. The complete draft NWRC study protocol (QA-2834) is attached as Appendix C.*

#### **1) Test guidelines and standards**

- EPA OPPTS 810.1000: Overview, Definitions, and General Considerations
- EPA Pesticide Assessment Guidelines - Subdivision G: Product Performance
- FIFRA Good Laboratory Practice Standards (GLPs; 40 CFR 160)

#### **2) Pre-test and two-choice test diets**

**Pre-test diet (maintenance diet):** Commercial dry cat food - Brand X<sup>9</sup>

**Challenge diet:** Commercial dry cat food - Meow Mix® (The J.M. Smucker Co., Decatur, Alabama, USA)

**Toxic bait:** Fish-based bait for mongoose (active ingredient: 0.005%/50ppm diphacinone, CAS # 82-66-6)

**Manufacture, handling, and characterization of test diets:** The toxic fish-based bait for mongoose (0.005% diphacinone) will be manufactured at Animal Control Technologies (Australia) Pty Ltd in Somerton, Victoria, Australia (EPA Establishment No.: 091731-AUS-001). The batch manufacturing sheet for the toxic bait will be included in the final report. A temperature and relative humidity data logger will accompany the bait during shipment to the USDA NWRC Hawaii Field Station in Hilo, Hawaii.

The pre-test diet (Brand X dry cat food) and the challenge diet (Meow Mix dry cat food) will be purchased from a commercial pet food supplier.

The pre-test diet, challenge diet, and toxic bait will be stored separately at the USDA NWRC Hawaii Field Station. A temperature and relative humidity data logger will accompany each diet in storage.

The % w/w diphacinone in the toxic bait will be characterized by the NWRC Chemistry Unit in Fort Collins, Colorado in accordance with FIFRA GLP Standards under a separate protocol. A GLP Certificate of Analysis for the toxic bait will be included in the final report.

#### **3) Test animals**

<sup>9</sup> To be determined



Species and type	Small Indian mongoose ( <i>Herpestes auropunctatus</i> ), wild caught
Numbers and sex	Treated group: 20 (10M:10F) Control group: 20 (10M:10F)
Body weight range 1 week prior to trial	Males: 600-800 grams Females: 350-500 grams
Age	Adult
Source	Wild caught in forested habitat, Hilo, Hawaii

#### 4) Pre-test period procedures

**Individual identification:** Upon arrival at the NWRC Hawaii Field Station, each animal will be assigned an individual ID number.

**Animal housing:** Animals will be individually housed in 42L x 61W x 64D cm grated-bottom stainless steel modified rabbit-type cages (3904 cm sq. floor area) in the same laboratory room used for the two-choice efficacy trial. Each cage will be assigned a unique number that corresponds with the animal's ID number.

**Environmental conditions:** Laboratory environmental conditions will be within the range of 20-25 °C, with a light cycle of 12 hrs light:12 hrs dark (lights on from 0600 to 1800 hours). The NWRC Hawaii Field Station laboratories typically range from 75 to 90% humidity; similar to what mongooses are naturally exposed to in the wild.

**Acclimation period:** Animals will be acclimated to the laboratory conditions for at least 7 consecutive days (no more than 28 consecutive days) during the pre-test period.

During the last 3 days of the acclimation period, animals will be provided food in two identical SS shield feeders on opposite sides of the front of their cages, which will be used to feed the animals for the rest of the trial. Instead of metal or ceramic dish feeders, disposable plastic feeder cups within metal feeder shields (Figure 2; Unifab, Portage, MI, USA), or similar types will be used. These feeder systems have custom shield sizes to prevent animals from nesting in feeder dishes. They are also designed to reduce spillage and cross contamination of the two diets offered to the treated group, which makes weighing uneaten food much easier.

**Pre-test period diet:** Animals will have ad libitum access to the pre-test diet (approximately 70 grams per day), supplemented by 50 grams of previously frozen raw chicken parts once every 4 days (Table 1). Based on past study experiences maintaining wild-caught mongooses in captivity, some caged mongooses will not feed sufficiently on the commercial diet (dry cat food) alone over multiple days and require supplementation with meat products to maintain body weight and health.

**Drinking water:** Animals will have ad libitum access to drinking water (tap water treated and tested for human consumption).

**Health and mortality checks:** Each animal will be checked for visible symptoms or mortality in person at least once each afternoon/evening.

**Animal weighing:** Animals will all be weighed on the same day within 3 days prior to the beginning of the test period.

## **5) Two-choice test period procedures**

**Assignment to treatment groups:** Animals will be randomly assigned to the two treatment groups, stratified by sex, while ensuring that there is no size bias among groups. Method of randomization will be recorded and reported.

**Two-choice test period duration:** The two-choice test period will last 3 consecutive days even if all animals in the treated group succumb to the toxic bait before the end of the two-choice test period.

**Test diet feeder locations:** Each morning of two-choice test period, each animal in the treated group will be offered two feeder dishes, one containing the toxic bait and the other containing the challenge diet (Table 1). The two diets will be offered in separate identical SS shield feeders on opposite sides of the front of the cage. Each day of the two-choice test period, the positions of the two feeders in each cage will be reversed from their positions the previous day to offset possible feeding position preferences of mongooses. The control group will be provided with challenge diet in both feeders each day. The location of the two feeders at the front of each cage will also be switched each day.

**Test diet amounts:** At least 70 grams of toxic bait and 70 grams of challenge diet will be available to each individual in the treated group per day during the two-choice phase. At least 70 grams of the challenge diet will be offered to each animal in the control group in each of two identical feeders (70 grams each). No supplemental raw chicken will be offered during the two-choice test period.

**Drinking water:** Water will be provided ad libitum throughout the test period.

**Daily consumption measurements:** The amount of each diet consumed by each animal will be measured approximately every 24 hours during the two-choice test period. The recorded amount consumed will not include any spilled food, which will be collected and dried (if necessary) before weighing.

After daily food weighings, the test diet in each feeder will be completely replaced with fresh test diet of the same type (70 grams per feeder). The feeder will be cleaned first if it becomes fouled by urine or feces.

**Health and mortality checks:** Animal health and mortalities will be checked twice daily between 8:00am-11:00am and 3:00pm-4:00pm throughout the test period, and symptoms will be recorded in the animal health log. Dead mongooses will be removed daily or more frequently as observed, weighed, placed in individual labeled (date, weight, sex) plastic bags and stored in the

freezer. There will be no euthanasia performed during the 20-day combined two-choice test and post-test periods.

***Trial termination criteria:*** If greater than 10% mortality occurs in the control group during the 20-day combined two-choice test and post-test periods, the trial will be discontinued and the results negated.

## 6) Post-test period procedures

***Post-test period duration:*** The post-test period will be maintained for 17 days for all surviving animals. We will continue to monitor control mongooses for the entire combined 20-day two-choice test and post-test periods, regardless of whether all of the treated group animals perish before that time.

***Challenge diet amounts:*** Each morning of the post-test period, the two feeders in each cage in both the treated and control groups filled with 70 grams of challenge diet. We will resume offering a supplement of 50 g of raw chicken parts every four days during this phase.

***Drinking water:*** Drinking water will be provided ad libitum throughout the post-test period.

***Daily consumption measurements:*** The amount of challenge diet and supplemental chicken consumed by each animal will be measured approximately every 24 hours during the post-test period. The recorded amount consumed will not include any spilled food, which will be collected and dried (if necessary) before weighing.

After daily food weighings, the challenge diet in each feeder will be completely replaced with fresh challenge diet (70 grams per feeder). The feeder will be cleaned first if it becomes fouled by urine or feces.

***Health and mortality checks:*** Animal health and mortalities will be checked twice daily between 8:00am-11:00am and 3:00pm-4:00pm throughout the test period, and symptoms will be recorded in the animal health log. Dead mongooses will be removed daily or more frequently as observed, weighed, placed in individual labeled (date, weight, sex) plastic bags and stored in the freezer. There will be no euthanasia performed during the 20-day combined two-choice test and post-test periods.

***Trial termination criteria:*** If greater than 10% mortality occurs in the control group during the 20-day combined two-choice test and post-test periods, the trial will be discontinued and the results negated.

On the day following the post-test period, all remaining mongooses will be humanely euthanized, weighed and carcasses placed in labeled plastic bags and stored in the freezer (-20° F).

## 7) Reporting and evaluation of results

**Final report contents:** All individual data and summary statistics (e.g., means and standard deviations) on bodyweights, bodyweight changes, food consumption, symptoms observed during the twice daily health checks, day of the trial that death occurred, and death rate per treatment group will be provided in the final report. Copies of all “raw” data sheets will also be appended to the final report.

**Minimum efficacy criteria:** The efficacy of the toxic bait will be considered acceptable if all of the following conditions are met:

- $\geq 33\%$  of the total food consumed by the treated group during the two-choice test period was the toxic bait.
- $\geq 90\%$  of the treated group died during the 20-day combined two-choice test and post-test periods.
- $\leq 10\%$  of the control group died during the 20-day combined two-choice test and post-test periods.

**Table 1.**

<b>Trial period</b>	<b>Feeders and diet</b>
Pre-test (acclimation) period (7-28 days)	Both treated and control groups: <ul style="list-style-type: none"> <li>• In-cage feed hopper of pre-test diet, supplemented with raw chicken once every 4 days</li> <li>• <i>During the last 3 days of the pre-test period:</i> 2 identical SS shield feeders of pre-test diet, supplemented with raw chicken once on the day prior to the two-choice test period</li> </ul>
Two-choice test period (3 days)	Treated group: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders containing either toxic bait or challenge diet, alternating the location of the toxic bait and challenge diet feeders each day</li> </ul> Control group: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders, both containing challenge diet, alternating the location of the two feeders each day</li> </ul>
Post-test monitoring period (17 days)	Both treated and control groups: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders, both containing challenge diet</li> </ul>
End of trial	



**Figure 1.** Custom stainless steel feeder shield (Unifab, Kalamazoo, MI).

### Part III. Budget

<b>Category</b>	<b>Amount</b>
Salary	\$12,307.85
Benefits	\$2,251.79
Supplies	\$2,168.14
<b>Total Direct Expense</b>	<b>\$16,727.78</b>
Overhead	\$1,672.76
<b>TOTAL</b>	<b>\$18,400.54</b>

Expenses for additional salaries, materials, vehicles, facilities, shipping, etc., including future completion of the study, are paid by WS-NWRC.

## **Appendix A: QA-2832 Final Report**

*This final version of the QA-2832 report includes data on timing of feeding bouts that were not yet ready for inclusion in the FY2018 Final Report to HISC. These results have also been presented and adapted for publication in the Proceedings of the 29<sup>th</sup> Vertebrate Pest Conference (currently in review).*

# Development and Testing Of a Matrix for Mongoose Toxic Bait: Nontoxic Bait Acceptance Cage Trials

Shane R. Siers<sup>1\*</sup>, Robert T. Sugihara<sup>1†</sup>, Emily W. Ruell<sup>2</sup>, I. L. Leinbach<sup>1</sup>, Daniel Sedgwick<sup>1</sup>, and Chris N. Niebuhr<sup>1,3</sup>

February 10, 2020

<sup>1</sup> USDA APHIS Wildlife Services, National Wildlife Research Center, Hawaii Field Station, Hilo, HI

<sup>2</sup> USDA APHIS Wildlife Services, National Wildlife Research Center, Registration Unit, Fort Collins, CO

<sup>3</sup> Current affiliation: Manaaki Whenua Landcare Research, Lincoln, New Zealand

† Study director

\* Corresponding author: shane.r.siers@usda.gov

## Sponsor:

Hawaii Invasive Species Council

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## Executive Summary

The only pesticide currently registered for mongoose control is a product developed for rats that consists of a hard pressed cereal bait block. Although the active ingredient (diphacinone) is known to be highly effective for mongoose, previous studies indicate that carnivorous and

omnivorous mongooses do not readily consume the hard bait matrix designed for gnawing rodents. A palatable bait matrix with a consistency more appropriate to mongoose dentition and feeding behavior will be required to develop a more effective mongoose pesticide.

We evaluated the acceptance and consumption of nontoxic versions of four candidate bait matrices: FOXECUTE<sup>®</sup> and FOXSHIELD<sup>®</sup> (Animal Control Technologies (Australia) (ACTA)); HOGGONE<sup>®</sup> (ACTA); and a potted pork shoulder loaf containing artificial dead mouse scent developed by WS-NWRC as a bait for invasive brown treesnakes (hereafter 'BTS bait').

After an acclimation period, we offered test groups of six mongooses one of the candidate bait matrices alongside dry dog kibble dog food as a challenge diet for five days. Because the potential active ingredients PAPP and SN require accumulation of the toxicant within a relatively brief period of time to affect lethal toxicity before they are metabolized, we conditioned mongooses to feeding within only a four-hour window rather than slowly sampling the bait throughout the night. Compared to ad libitum food access, limited availability of palatable food items is more representative of mongoose encounters with unreliable food sources in the field. We estimated rate and amount of consumption through review of time-lapse photography of feeding trials, and measured total consumption by weighing uneaten portions of bait.

From the first day offered, mongooses readily consumed ample amounts of all four bait matrices and consumed almost no challenge diet, with few exceptions. Overall, consumption was highest and





most consistent with the BTS bait.

Although this trial did not clearly discriminate an optimal bait matrix, this result is highly encouraging in that we have multiple palatable options. The final selection will be based on other characteristics of the bait matrix such as longevity in the field, compatibility with the selected toxicant, and ease of manufacture, storage, and use. We provide an overview of some of these characteristics for each candidate bait type.

## Introduction

Introduced small Indian mongooses (*Herpestes auropunctatus*) are serious predators of native wetland, seabird and upland forest avian species in the Hawaiian Islands (Hays and Conant 2007), as well as in other introduction sites worldwide (Nellis and Everard 1983; Yamada and Sugimura 2004). Mongooses are well established across most of the main Hawaiian Islands (Hawaii, Oahu, Maui and Molokai) where they pose a threat to the eggs and nestlings of native ground-nesting birds (Hays and Conant 2007). The threat of accidental or intentional introductions to other mongoose-free islands in the Hawaiian chain (e.g. Kauai, Lanai) and other Pacific locations highlights the need for a comprehensive menu of control techniques, including attractive and palatable baits and effective toxicants, to quickly respond to reported sightings or incipient mongoose populations under a diversity of scenarios (Phillips and Lucey 2016). Mongooses also present a health risk to humans as hosts of leptospirosis in Hawaii (Wong et al. 2012) and the Caribbean (Everard 1976), and as a rabies reservoir on several islands in the Caribbean (Zieger et al. 2014).

Eradication of introduced mammals is a powerful conservation tool (Howard et al. 2007); however, mongoose eradication has been attempted only on few occasions and with limited success. A known total of eight eradication campaigns and many control campaigns have been conducted to remove or reduce island mongoose populations (Barun et al. 2011). However, even with their limited scope, these attempts probably delayed or prevented further declines or even extirpations of native species. Very few teams have the technical expertise to remove mongooses successfully, even

from small islands. Such lack of expertise is reflected by past failures and little progress beyond local trapping control programs. In Amami-Oshima, Japan, over 10 years of intensive trapping reduced mongoose populations island-wide; however, alternative methods such as toxicants are being considered and tested to eradicate remnant mongooses in difficult-to-trap areas. In Hawaii, live-traps (Tomahawk Live Trap, Tomahawk, WI) and registered 50 ppm diphacinone wax block baits applied within bait stations are employed (SLN No. HI-980005; Smith et al. 2000, Barun et al. 2011). However, these methods have been less successful in areas with low mongoose density or high alternate prey density.

USDA WS-NWRC Hawaii Field Station researchers have conducted field studies evaluating various potential lures, attractants, and bait types (Pitt et al. 2015). Mongooses in this study foraged over a wide area (mean home range estimates were 21.9 and 28.8 ha at two study sites), and readily investigated the various novel food baits, including fish, beef and egg-baited stations with revisits over multiple days. However, long-lasting lures and palatable baits still need to be developed and trialed in the field.

A recent WS-NWRC cage trial of several candidate toxicants, including commercial rodenticide formulations, novel toxicants (sodium nitrite [SN] and para-aminopropiophenone [PAPP]), and minced-chicken formulations with diphacinone, demonstrated potential for development of a highly-effective toxic bait for mongoose control (Sugihara et al. 2017). These findings also indicated that the relative inefficacy of the commercial rodenticide formulations was likely due to the hard consistency of grain-based pellets and blocks which are not appropriate to the dentition and feeding modes of mongooses. Additionally, a toxicant registration evaluation was recently produced for mongooses in Hawaii by WS-NWRC (Ruell et al. 2019). The results of this review indicate that sodium nitrite, PAPP, diphacinone, and bromethalin all have potential to be registered as toxicants for mongoose control for use in bait stations if suitable toxicant/bait matrix combinations can be identified, with a diphacinone bait being the least expensive and fastest to register. A diphacinone bait could also potentially be registered for limited uses outside of bait stations.

Development of an effective mongoose bait will require a softer, palatable matrix that can be paired with an effective toxicant.

## Objective

In this pilot phase of mongoose toxic bait development, we evaluated bait acceptance of selected nontoxic bait matrices for mongooses, a necessary first step before incorporating toxicants. By identifying potential nontoxic bait matrices that are palatable to mongooses and ruling out those that are not, we ultimately minimized the number of trials, and thus animals, necessary to conduct subsequent palatability trials involving various combinations of bait matrices and toxicants. The objective of this pilot phase is to simply gauge which of the candidate matrices have adequate bait acceptance rate (i.e., are consumed in sufficient amounts) to warrant future consideration as a toxicant matrix.

We assessed acceptability and consumption of four nontoxic versions of the following bait matrices (Figure 1):

- Nontoxic FOXECUTE® and FOXSHIELD® are semi-soft blocks of meat- and fish-flavored bait, respectively, produced by Animal Control Technologies (Australia) (ACTA). Commercial versions in Australia have a sausage-like casing and are formulated with PAPP for invasive fox control.
- Nontoxic HOGGONE® (ACTA) is a peanut paste-based bait. A 10% SN version of HOGGONE was recently registered in Australia for control of feral swine. A modified HOGGONE formulated with 5% SN is currently in development for feral swine control in the US.
- ‘BTS bait’ is a processed pork shoulder loaf formulated with synthetic lipids mimicking the scent profile of dead mice. This product was developed by WS-NWRC as a cost-effective alternative to dead newborn mice as a vehicle to deliver acetaminophen to invasive brown treesnakes.

## Methods

### Mongoose capture

Wild small Indian mongooses were trapped in Hilo, HI and surrounding areas, and transported to and individually housed in the WS-NWRC research facility per standard internal protocols (SOP AC 005.00). Upon arrival, sex and body mass were recorded for each animal.

Animals were dusted for ectoparasites with Drione® (1.0% pyrethrin) before entering the test facility. A bellows duster was used to lightly coat the nape and dorsal areas of the mongooses, avoiding the eyes, nose, and mouth, while still in the trap.

Any animal with injuries, sustained aggressive behavior, or poor body condition (pelage mangle, worn or missing teeth) were immediately euthanized by carbon dioxide inhalation (SOP AC/HI 002.01). Twenty four (24) animals were used, including three (3) of each sex for each of the four (4) nontoxic bait matrices trialed. An additional 4-6 mongooses were housed as spare animals to replace animals deemed unfit for inclusion in trials. We randomly assigned mongooses to test groups while ensuring a relatively equal sex ratio within each group.

### Housing

Mongooses were held in stainless steel rabbit cages (Allentown Caging Equipment Co., Inc., Allentown, NJ), with each individual cage measuring 42 cm tall x 61 cm wide x 64 cm deep (Fig.3) which allowed the full range of natural movement. Mongooses had ad libitum access to water in ball-stoppered bottles attached to the front of the cage at all times throughout all phases.

### Acclimation and conditioning phase

Mongooses were subject to an acclimation period of 5–7 days prior to feeding trials. The test room was maintained at 24–25° C and 12:12 h light:dark cycle during the trials. For the first 48 hours of captivity, mongooses had ad libitum access to a maintenance diet (dry cat food pellets) until they exhibited consumption; animals that did not consume cat food during this window were not included in the study. Once they began



Figure 1: Nontoxic versions of candidate bait matrices for acceptance and consumption trials. Left to right: FOXSHIELD (ACTA), FOXECUTE (ACTA), HOGGONE (ACTA), and pork loaf BTS bait with artificial mouse carrion scent (WS-NWRC).

consuming the maintenance diet, mongooses were conditioned to receiving access to their daily ration within a limited time window each morning (4 hours, 0800-1200 h) to simulate infrequent food item encounters in the field, such as natural prey or baits in bait stations. This limited window for consumption is also important for judging whether a bait is a suitable matrix for SN or PAPP, because their modes of action require consuming enough of the toxicant over a short enough window to achieve a lethal effect. Food was provided in the morning, while cage cleaning and maintenance occurred in the afternoon to minimize stress while food is available.

To mimic the presentation of toxic bait in the field and to prevent spillage from falling through the grated cage floor, we used Protecta LP® bait stations (Bell Laboratories, Inc., Madison, WI) as feed trays for all phases of this study (Figure 2). We modified bait stations by removing the top cover to allow for monitoring of consumption by video recording.

### Trial phase

We evaluated acceptance and consumption of nontoxic bait matrices via two-choice feeding trials. Test baits were provided along with an equal amount, by mass, of dry dog kibble (Doggy Bag™) challenge diet (different than the dry cat kibble maintenance offered during the acclimation phase). To mimic bait block presentation in bait stations, we secured the nontoxic FOXSHIELD, FOXECUTE, and BTS bait within bait stations on



Figure 2: Modified Protecta LP bait station used as feeding tray. Blocks or loafs of the nontoxic bait matrix (test diet) were secured on the horizontal wire rod included with the bait station. Dry dog kibble challenge diet was offered in the tray below the test diet. Nontoxic HOGGONE paste was provided alongside the challenge diet in the tray.

the wire rods provided with the commercially available rodenticide bait stations (Figure 1, right); these rods are intended to prevent removal of the bait block from the bait station. HOGGONE, a paste, was placed on the bait station floor in the tray area intended for loose baits (e.g. pellets). The dry dog kibble challenge diet was also offered in the floor tray directly beneath the rod-mounted baits or beside the paste bait. For each trial, we offered 70 g each of test and challenge diet at the same time. We estimated 70 g as the upper range of what we would expect could be consumed by a mongoose in a single feeding. We conducted each trial in the morning, with baits available

for the same 4-hour window allowed during the acclimation period, approximately 0800 to 1200. After each exposure period, we removed the bait stations and test baits. We weighed any uneaten or spilled test or challenge diet remaining in the bait station or on the cage floor or excreta collection tray to assess consumption.

Due to variation in humidity levels in the animal testing room, both the test and challenge diets were expected to gain or lose small amounts of moisture each day during the exposure period. Therefore, two samples each diet were weighed and placed in empty mongoose cages similar to those used for the trials. The moisture control samples were exposed to the same environmental conditions in the same room as the test animals during the exposure period, and were weighed at the same time as the food remaining after the exposure period. The weights of diets offered each day were then adjusted by multiplying a correction factor calculated as the final weight of the environmental control sample divided by the initial weight. The corrected amount offered at the start of the exposure period was used to calculate amount eaten from each feeder (i.e., amount eaten = corrected amount offered – amount remaining).

We repeated feeding trials, using the same test diet for each treatment group, for 5 days. If any animal exhibited signs of lethargy and/or illness, or were not consuming any food during the trial phase, that animal was offered small amounts of raw chicken pieces as a diet supplement. If any animal continued to show signs of inappetence or distress, it was euthanized and not replaced.

The order of treatment group trials was randomized, with nontoxic FOXSHIELD and HOGGONE trials commencing 29 April 2019, nontoxic FOXECUTE commencing 6 May 2019, and nontoxic BTS bait on 13 May 2019.

### Consumption rate monitoring

We monitored frequency and duration of feeding events by video recording using GoPro® cameras (Hero 5 Black and Hero 7 Silver models; San Mateo, CA). We mounted cameras approximately 23-30 cm directly above the bait on a flat aluminum bar secured to the vertical rear wall of the bait station (Figure 3, left). From this perspective, the cameras could capture the full view of the test

bait and challenge diet and visitation/sampling by the mongoose. To accustom mongooses to the presence of cameras during the trial phase, we painted wooden blocks black to mimic cameras and mounted them in the same position during the acclimation phase. Because of battery capacity limitations, the Hero 5 Black models did not capture the entirety of each feeding period and were used to record only the nontoxic HOGGONE feeding trials.

We analyzed videos of each feeding trial and recorded the duration of each feeding event and visually estimated the amount of bait matrix that was consumed during each event. Videos were recorded at 2 frames/sec and rendered at 29 frames/sec. We calculated the real-time duration of each feeding event using the formula  $((x*29)/2)$ , where  $x$  = video duration of feeding event in seconds. We visually estimated the amount of bait matrix consumed during any given feeding event as a percentage of the total mass that was offered. We obtained the actual total mass eaten by weighing the remaining diet at the end of the exposure period. We used the estimated percentages eaten from observations and the measured total consumption to estimate the mass of bait eaten during each feeding event.

## Results

Acceptance and consumption of all test baits was high. All baits were very highly preferred over the dry dog kibble challenge diet, with many mongooses consuming none of the dry dog kibble on most days (Tables 1-4).

Consumption rates estimated from video observations are depicted in Figures 4 a-d. These represent the maximum amount of the bait matrix that was consumed during any 30- or 60-minute sliding window of time throughout each 4-hour feeding session. The entire amount consumed during the feeding session is also depicted. The dosage of active ingredient consumed during any such period can be estimated from the amount of matrix consumed, the concentration of the toxicant in the matrix, and the mass of the mongoose.

Table 1: Consumption values (g) of nontoxic FOXECUTE test diet (Trt) and challenge diet (Ch), by individual, day, and overall, for six mongooses. Pref = preference ratio for test:challenge diet over all five days of feeding. "Inf." = Infinite, a preference ratio is not quantifiable when consumption of one of the options was zero.

ID #	Sex	Diet	Day 1	Day 2	Day 3	Day 4	Day 5	Avg	Pref
119	F	Trt	20.21	18.08	16.62	16.95	18.68	18.11	76:1
		Ch	0.00	0.01	0.70	0.33	0.16	0.24	
132	M	Trt	2.60	0.77	0.12	*	*	1.17	*
		Ch	0.00	0.00	0.46	*	*	0.15	
122	M	Trt	11.36	19.19	25.08	20.66	24.04	20.07	18:1
		Ch	0.00	2.19	0.99	1.18	1.28	1.13	
131	F	Trt	19.42	24.00	25.72	14.82	18.12	20.42	19:1
		Ch	3.54	0.34	0.47	0.09	0.87	1.06	
126	M	Trt	33.62	25.99	45.64	38.38	41.49	37.02	115:1
		Ch	0.00	0.24	0.88	0.00	0.49	0.32	
133	F	Trt	0.01	0.00	0.00	0.00	0.39	0.08	0.01:1
		Ch	10.67	12.49	7.52	6.71	7.03	8.88	
Average		Trt	14.54	14.67	18.86	18.16	20.54	16.14	46:1
		Ch	2.37	2.54	1.84	1.66	1.97	1.96	

\* Individuals that consistently failed to feed on either diet item were removed from the study and euthanized.

Table 2: Consumption values (g) of nontoxic FOXSHIELD test diet (Trt) and challenge diet (Ch), by individual, day, and overall, for six mongooses. Pref = preference ratio for test:challenge diet over all five days of feeding. "Inf." = Infinite, a preference ratio is not quantifiable when consumption of one of the options was zero.

ID #	Sex	Diet	Day 1	Day 2	Day 3	Day 4	Day 5	Avg	Pref
103	M	Trt	1.90	2.39	5.66	19.92	22.86	10.55	61:1
		Ch	0.00	0.00	0.00	0.64	0.22	0.17	
105	F	Trt	27.13	27.03	21.68	17.32	19.65	22.56	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
106	M	Trt	36.49	19.41	26.74	26.59	31.79	28.20	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
110	M	Trt	21.41	19.48	31.88	29.06	30.89	26.54	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
111	F	Trt	17.47	25.98	25.93	24.40	25.61	23.88	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
116	F	Trt	25.73	29.39	9.96	21.05	7.13	18.65	4.9:1
		Ch	0.00	0.00	7.08	2.85	8.95	3.77	
Average		Trt	21.69	20.62	20.31	23.06	22.99	21.73	>>33:1
		Ch	0.00	0.00	1.18	0.58	1.53	0.66	

Table 3: Consumption values (g) of nontoxic HOGGONE test diet (Trt) and challenge diet (Ch), by individual, day, and overall, for six mongooses. Pref = preference ratio for test:challenge diet over all five days of feeding. "Inf." = Infinite, a preference ratio is not quantifiable when consumption of one of the options was zero.

ID #	Sex	Diet	Day 1	Day 2	Day 3	Day 4	Day 5	Avg	Pref
102	M	Trt	10.24	16.35	20.82	17.66	18.87	16.79	6.8:1
		Ch	9.58	0.00	0.02	1.00	1.75	2.47	
107	F	Trt	5.86	11.66	8.10	8.44	6.32	8.07	8.7:1
		Ch	3.70	0.00	0.00	0.00	0.95	0.93	
108	M	Trt	36.90	24.66	28.14	19.13	17.36	25.24	120:1
		Ch	0.00	0.00	0.00	1.05	0.00	0.21	
109	F	Trt	8.66	6.12	0.28	*	*	5.02	*
		Ch	0.00	0.00	0.02	*	*	0.01	
114	F	Trt	8.56	12.69	9.55	12.74	12.85	11.28	22:1
		Ch	1.65	0.95	0.00	0.00	0.00	0.52	
115	M	Trt	11.93	14.66	13.63	17.45	16.80	14.89	8.0:1
		Ch	5.88	2.71	0.00	0.75	0.00	1.87	
Average		Trt	13.69	14.36	13.42	15.08	14.44	13.55	33:1
		Ch	3.47	0.61	0.01	0.56	0.54	1.00	

\* Individuals that consistently failed to feed on either diet item were removed from the study and euthanized.

Table 4: Consumption values (g) of nontoxic BTS bait test diet (Trt) and challenge diet (Ch), by individual, day, and overall, for six mongooses. Pref = preference ratio for test:challenge diet over all five days of feeding. "Inf." = Infinite, a preference ratio is not quantifiable when consumption of one of the options was zero.

ID #	Sex	Diet	Day 1	Day 2	Day 3	Day 4	Day 5	Avg	Pref
136	F	Trt	20.74	29.63	29.89	36.34	30.17	29.35	42:1
		Ch	1.84	1.63	0.00	0.00	0.00	0.69	
137	F	Trt	22.40	20.51	20.58	26.24	19.22	21.79	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
138	M	Trt	31.25	38.13	36.96	53.16	60.40	43.98	265:1
		Ch	0.00	0.00	0.03	0.79	0.00	0.17	
139	M	Trt	23.95	20.82	21.29	34.86	32.94	26.77	Inf.
		Ch	0.00	0.00	0.00	0.00	0.00	0.00	
140	F	Trt	17.65	19.78	19.62	22.10	25.42	20.91	238:1
		Ch	0.00	0.00	0.07	0.37	0.00	0.09	
144	M	Trt	36.34	39.41	50.30	48.57	53.91	45.71	144:1
		Ch	0.00	0.00	0.77	0.82	0.00	0.32	
Average		Trt	25.39	28.05	29.77	36.88	37.01	31.42	>150:1
		Ch	0.31	0.27	0.15	0.33	0.00	0.21	





Figure 3: Left: Bait station serving as feeding tray, with aluminum bar as camera mount; in this image, a painted wooden block serves as a surrogate to acclimate mongooses to the presence of a camera during the trial phase. Right: Camera field of view, with the test bait (nontoxic FOXECUTE) pinned on the bait station wire bar and dog kibble challenge diet within the tray underneath.

## Discussion

Two mongooses, 1M:1F, were removed from the study due to prolonged failure to feed on either diet offered. For what little they did eat, both preferred their test diet (nontoxic FOXECUTE and HOGGONE) over the challenge diet. Given the reliable consumption by others in their treatment groups, we believe that their failure to thrive was independent of the treatment and likely due to physiological or psychological factors, and should not reflect poorly on the suitability of the bait matrix.

Of the 24 test animals, only one, a female in the nontoxic FOXECUTE treatment group, preferred the dry dog kibble challenge diet and ate almost no treatment diet. Preference ratios of the other animals in the same test group ranged from 18:1 to 115:1, indicating this individual as an outlier. Again, it appears unlikely that this anomaly indicated reduced suitability of nontoxic FOXECUTE as a bait matrix.

Excluding these three outliers, average daily consumption of baits, ranked from highest to lowest, were: nontoxic BTS bait ( $31\text{g} \pm 11.75\text{ SD}$ ) > nontoxic FOXECUTE ( $24\text{g} \pm 13.01$ ) > nontoxic FOXSHIELD ( $22\text{g} \pm 8.63$ ) > nontoxic HOGGONE

( $15\text{g} \pm 7.40$ ). The highest exceeded the lowest by a factor of two.

Our results indicate that we are in the fortunate circumstance of having several bait matrix options that are palatable to wild-caught mongooses. The selection of a bait matrix for formulation in a registered product will likely be on the basis of other characteristics such as longevity in the field, compatibility with the selected toxicant, and ease of manufacture, storage, and use. The four candidate toxicants for pairing with a preferred bait matrix are diphacinone, bromethalin, SN, and PAPP (Ruell et al. 2019). Below we discuss our results in light of other matrix and toxicant characteristics:

**Nontoxic FOXECUTE and FOXSHIELD** – Both products performed well in feeding trials. Nontoxic FOXECUTE was preferred to the dog kibble by a factor of 46, while the preference ratio for nontoxic FOXSHIELD was inestimable in that four of the six mongooses in the treatment group ate no challenge diet and fed exclusively on FOXSHIELD. However, average daily consumption of FOXECUTE was slightly higher, though not likely significantly, than FOXSHIELD. FOXECUTE is

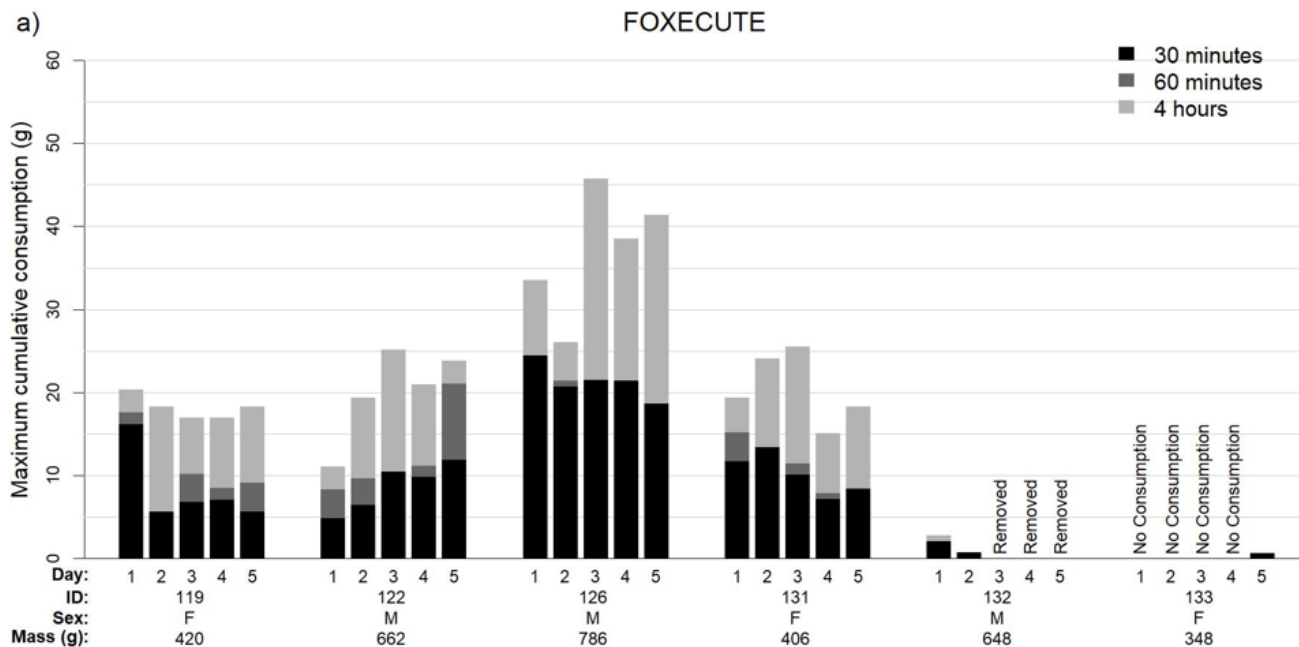


Figure 4: Time-bound consumption rates of nontoxic FOXECUTE estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

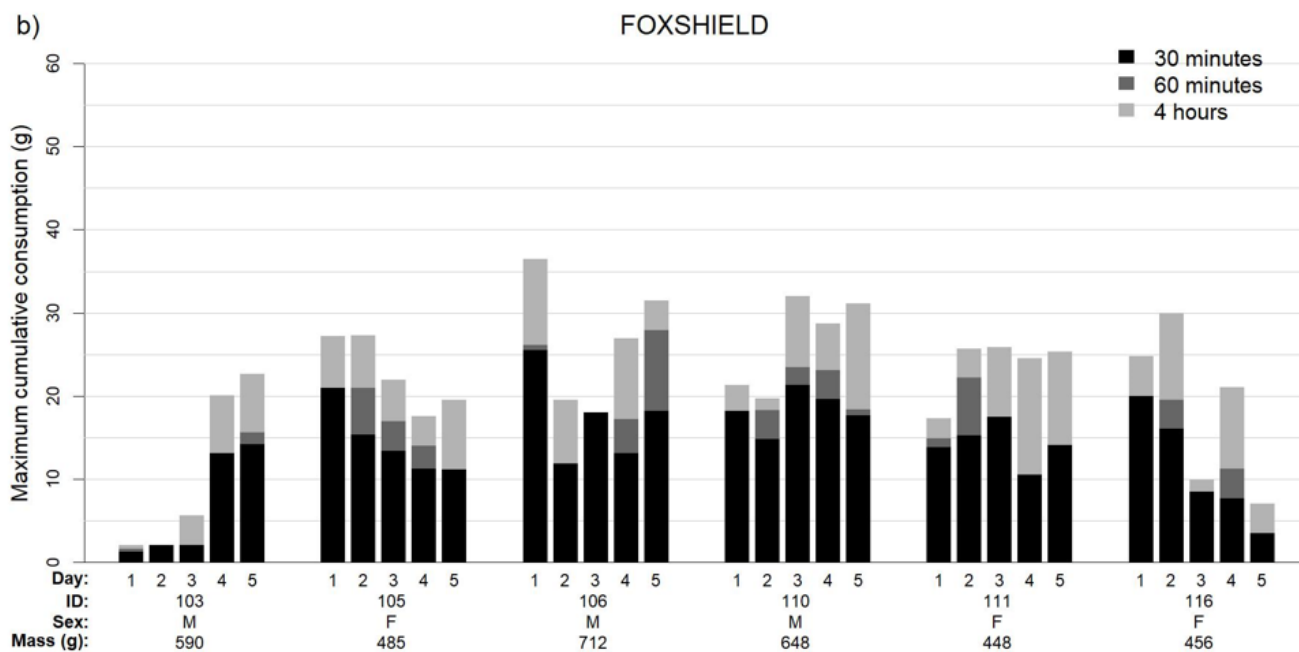


Figure 5: Time-bound consumption rates of nontoxic FOXSHIELD estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.



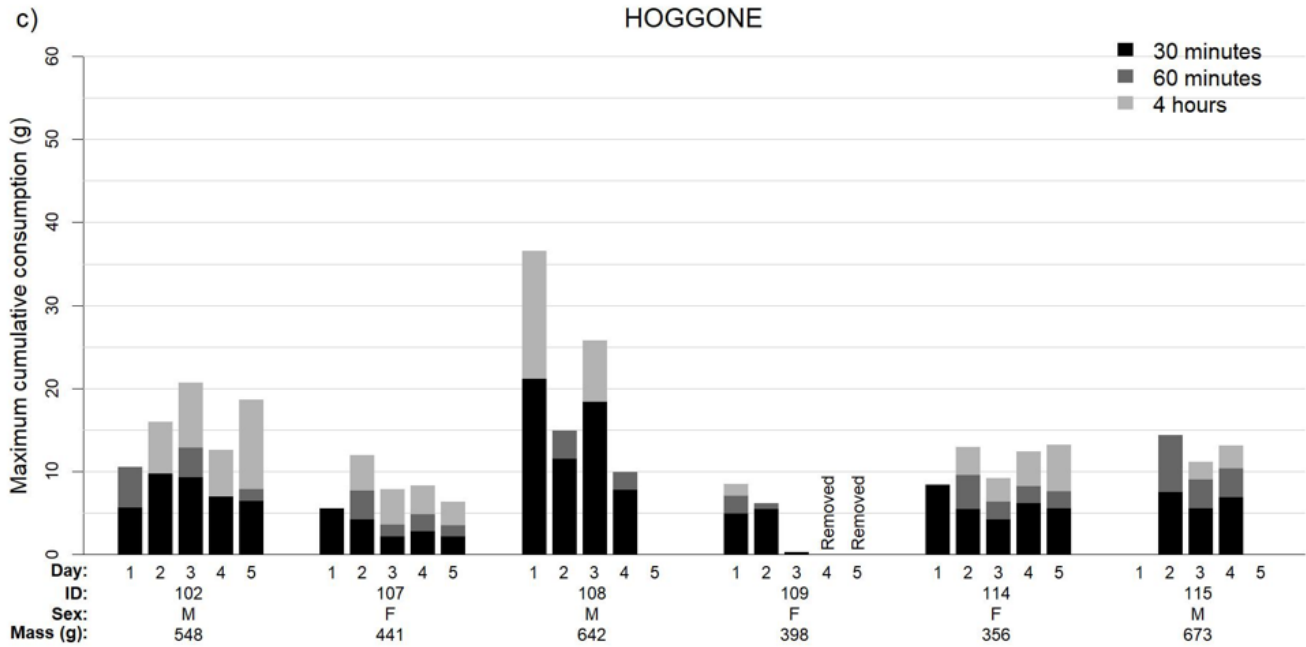


Figure 6: Time-bound consumption rates of nontoxic HOGGONE estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

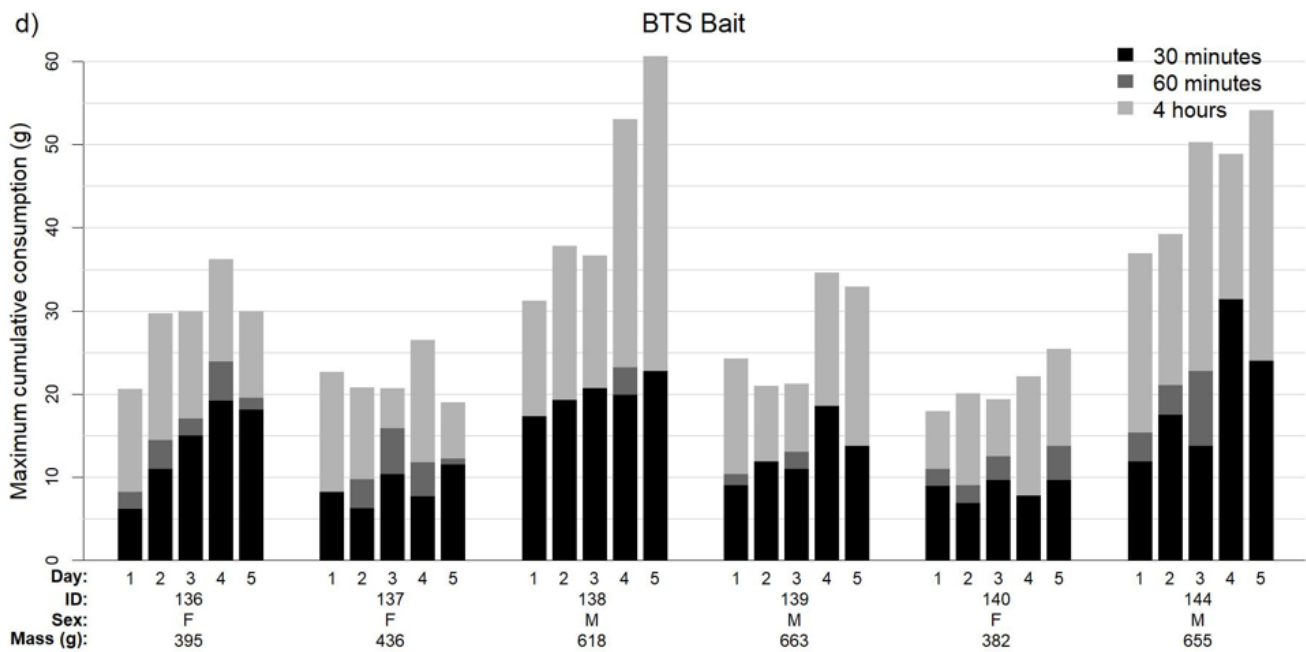


Figure 7: Time-bound consumption rates of nontoxic BTS bait estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

beef flavored, while FOXSHIELD is fish flavored. Fish products (sardines, oils) are routinely used as mongoose trap baits and lures and have been shown to be very attractive to mongooses, with extended attractiveness to lure mongooses from afar. Due to regulation of importation of animal products into the United States from Australia, the fish-flavored FOXSHIELD would likely have a lower barrier to importation. Although both products will require import permits from USDA APHIS Veterinary Services, the import of FOXECUTE for commercial distribution and use would likely be subject to additional livestock disease status certification requirements. Both baits are commercially formulated in Australia with PAPP as the active ingredient. There are no registered PAPP pesticide products in the United States and the barriers to registration are the highest of the candidate toxicants we consider (Ruell et al. 2019). These baits are not likely to be easy to formulate with SN, because of their moisture content and the current inability to reliably microencapsulate SN. Current microencapsulation formulations quickly degrade when exposed to moisture, exposing the sodium (causing high saltiness) and causing the release of noxious nitric oxides. The manufacturer (ACTA) does not currently formulate any products containing diphacinone or bromethalin. It is currently undetermined whether ACTA would invest in the equipment and regulatory approvals required to incorporate new toxicants into these matrices for a relatively niche application like mongooses. Thus, a second manufacturing step in the U.S. may be required. As for field usability, FOXECUTE and FOXSHIELD are currently in field use for fox control in Australia, and are formed in easily-handled discrete units and likely have favorable storage and longevity characteristics that would make them highly suitable as a matrix for a mongoose bait.

**Nontoxic HOGGONE** – Although preferred over dry dog kibble by a factor of 33, nontoxic HOGGONE had the lowest average daily consumption at 15 g. This might not be surprising; while the other baits are meat based or flavored formulations designed for carnivores, HOGGONE is based on peanut and cereal products which would probably be considered less attractive to a carnivorous mammal. Typically formulated

with SN for feral swine control, the amount and rate of consumption are important in achieving sufficient circulating levels of toxicant to achieve lethal intoxication before being metabolized out of the system. Nontoxic HOGGONE had some of the lowest time-bound and overall consumption rates, suggesting that mongooses would be somewhat less likely to achieve a sufficient circulating dosage to affect lethal intoxication than with other products. This could potentially be overcome by a higher concentration of toxicant in the matrix. Although SN is not an active ingredient in any registered pesticides in the U.S., USDA and collaborators have generated or contracted all of the registration data required for registration of SN as part of the development of HOGGONE as a toxic bait for feral swine (Ruell et al. 2019). If HOGGONE is registered in the U.S. for feral swine, it could be relatively easy to register the same formulation for mongooses. As a matter of practicality, HOGGONE presented the lowest ease of use in our trials. Being a paste, residues were fairly resistant to easy cleaning of bait stations. Reliable formulation of HOGGONE is troubled by the same SN encapsulation difficulties as mentioned above. Likewise, as an ACTA product, availability of the HOGGONE paste matrix formulated with diphacinone or bromethalin is questionable and may require a secondary manufacturing step in the U.S.

**BTS bait** – In our trials, mongoose consumed the WS-NWRC pork loaf with artificial mouse carrion scent most reliably and copiously at an average daily consumption of >30 g. The intent of the mouse scent is to act as an attractant to draw the nuisance predator to the bait; it has not yet been evaluated whether the mouse scent affects palatability to mongooses. It is clear that palatability with the scent is not an issue, and future determinations of whether to incur the additional expense of the mouse scent will depend on whether the scent draws mongooses to the bait stations from further away. This bait matrix is currently experimental and being manufactured in small batches at the WS-NWRC Hawaii Field Station in Hilo. Manufacture involves grinding and mixing of pork shoulder and other constituents, then sealing and cooking loaves within a foil pouch. As prepared, pouches

of bait are shelf-stable. Field stability has not yet been evaluated, though studies are underway. As currently produced, convenience of use in the field may not be optimal because the pork loaf, of a consistency very similar to the SPAM® (Hormel Foods Corporation) potted meat product, must be removed from the pouch and manually cut into shapes and amounts suitable for deployment in bait stations. Slightly wet with free-form fats and extruded scent lipids, frequent cleaning of hands and equipment will be required. If adopted as a mongoose bait matrix, the manufacturing process for the scented pork product may be adapted to produce sausage forms that would improve the ease of use. A major advantage is that this product requires no special equipment not available for commercial kitchens. Currently formulated in-house at WS-NWRC, we would be at liberty to incorporate any registered technical material as an active ingredient, provided that the Hawaii facility became registered as a pesticide-producing establishment and that the end product was registered as a pesticide. Beyond very small batches, manufacture could be transferred to the WS Pocatello Supply Depot, the primary WS facility for manufacturing and providing specialized wildlife damage management pesticides and other products that are not readily available from commercial sources.

Video monitoring of bait consumption provided additional insight into rates of consumption that would not have been available from only measuring remaining bait after the entire feeding period. This rate of consumption is particularly important with active ingredients that must be ingested in a large bolus because they metabolize quickly, such as PAPP and SN. Our results will be useful in evaluating the potential for lethal intoxication with one of these toxicants. Actual dosage would be a function of the feeding rate, the concentration of toxicant in the matrix, and the mass of the animal consuming the bait.

As a final usage note, the purpose of the pins or rods in a bait station are to prevent entire pesticide blocks from being removed from the bait station where they are exposed to consumption by nontarget species and are no longer available to other target species visiting the bait station. Suspended on horizontal rods, mongooses will

consume bait along the top surface of the bait; as more bait is consumed, the rod is exposed and the weight of unconsumed bait will keep the mass below the rod, which may sag and fall off leaving a large portion of the bait free to be carried off (Figure 5). We recommend that future bait station designs maintain blocks on vertical retainer rods, reducing the tendency of the mass of bait to remain in a position less accessible for feeding and to fall off of the rod in large quantities.

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**Appendix B: Ruell et al. 2019 Management of Biological Invasions 10(3):573–596**

*An uncorrected proof version of this manuscript was also included in the FY2018 Final Report to HISC. This attached manuscript is the final published version.*

## Research Article

**An evaluation of the registration and use prospects for four candidate toxicants for controlling invasive mongooses (*Herpestes javanicus auropunctatus*)**Emily W. Ruell<sup>1,\*</sup>, Chris N. Niebuhr<sup>2,3</sup>, Robert T. Sugihara<sup>2</sup> and Shane R. Siers<sup>2</sup><sup>1</sup>USDA, APHIS, WS, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA<sup>2</sup>USDA, APHIS, WS, National Wildlife Research Center, Hawaii Field Station, P.O. Box 10880, Hilo, HI 96721, USA<sup>3</sup>Present address: Landcare Research, PO Box 69040, Lincoln 7640, New ZealandAuthor e-mails: [emily.w.ruell@usda.gov](mailto:emily.w.ruell@usda.gov) (EWR), [niebuhrc@landcareresearch.co.nz](mailto:niebuhrc@landcareresearch.co.nz) (CNN), [robert.t.sugihara@usda.gov](mailto:robert.t.sugihara@usda.gov) (RTS), [shane.r.siers@usda.gov](mailto:shane.r.siers@usda.gov) (SRS)

\*Corresponding author

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## OPEN ACCESS

**Abstract**

The eradication or control of invasive small Indian mongooses from islands likely requires toxic baiting when removal by trapping proves insufficient. The one toxic bait currently registered for mongooses in the United States has relatively low palatability and efficacy for mongooses. Developing and registering a new pesticide can be very expensive, while funding for developing toxicants for mongooses is limited. Once registered, use of a toxic bait may be hindered by other factors, such as public opposition to an inhumane toxicant, poorer efficacy than expected, or if the toxic bait is difficult for applicators to apply or store. Therefore, we conducted a product feasibility assessment comparing the registration and use potential of toxic baits for mongooses containing either bromethalin, diphacinone, para-aminopropiophenone (PAPP), or sodium nitrite (SN). We estimated that a diphacinone bait would be the cheapest and fastest to register, and more application methods may be allowed compared to the others. On the negative side, we ranked diphacinone as the least humane toxicant of the four, largely due to a prolonged time to death following exposure and onset of symptoms. However, this interval also increases the probability that the antidote can be administered following an accidental exposure. If an alternative toxicant is required, use of a bromethalin, PAPP, or SN bait would likely be limited to bait stations or burrow baiting due to primary risks to non-target species. A bromethalin bait would be the cheapest and fastest to register of the three, particularly if a bait that is already commercially available proved efficacious for mongoose. However, we ranked bromethalin lower than PAPP or SN for overall humaneness. A PAPP bait would be slow and the most expensive to register. An SN bait would be challenging to formulate into a palatable bait with a reasonable shelf life. Although we focused on the U.S., mongooses are invasive in many parts of the world and the regulatory and use requirements for pesticides in other countries are generally comparable. In addition, our feasibility assessment can serve as a template or starting point for managers considering development of toxicant products for vertebrate pests.

**Key words:** humaneness, injurious wildlife, invasive species, mongoose, pest, pesticide, regulation, regulatory requirements, toxic baiting

**Introduction**

Many of the world's invasive vertebrate species were intentionally introduced by humans for biological pest control or for agricultural or commercial

reasons, but instead they caused native species extinctions, damaged ecosystems and crops, and spread diseases, resulting in large ecological and economic costs (Pimentel et al. 2000). Depending on the characteristics of the invasive species and location, successful control and eradication efforts against invasive populations may require the use of multiple management tools, including toxicants (Simberloff 2003).

The small Indian mongoose (*Herpestes javanicus auropunctatus* Hodgson, 1836; hereafter, mongoose) was intentionally introduced to the islands of Hawaii, Puerto Rico, and the United States Virgin Islands in the late 1800s through the early 1900s for the purpose of controlling rats (*Rattus* sp. Fischer, 1803) to protect sugarcane crops (Baldwin et al. 1952; Keith et al. 1989; Hays and Conant 2007; USFWS 2011; Berentsen et al. 2018). However, introduced mongooses decimated and continue to cause the decline of numerous native bird, mammal, amphibian, and reptile species on these islands (reviewed in Hays and Conant 2007; Barun et al. 2011; Berentsen et al. 2018). In addition, mongooses pose human health risks as some of the introduced populations carry and transmit zoonotic diseases, including rabies and leptospirosis (Everard et al. 1976; Everard and Everard 1992; Wong et al. 2012; Zieger et al. 2014; Berentsen et al. 2015). Pimentel et al. (2000) estimated that mongooses caused approximately \$50 million in damages each year in Puerto Rico and Hawaii.

Because of their impacts on native fauna and potential for disease transmission, mongooses were one of the first species listed as injurious wildlife under the Lacey Act of 1900 (18 U.S.C. §§ 42–43; USFWS 2017), which made it illegal to import, export, acquire, or transport mongooses in the U.S. or in any territory or possession of the U.S. (18 U.S.C. § 42). Laws in U.S. states and territories generally also prohibit the acquisition, possession, distribution, or release of any species classified as invasive, harmful, or injurious, including Hawaii (Hawaii Administrative Rules 13-124-3) and the Commonwealth of Puerto Rico (The New Wildlife Act of Puerto Rico, Law No. 241). The mongoose also makes the list of the top 100 of the world's worst invasive alien species from the Global Invasive Species Database from the International Union for Conservation of Nature (IUCN 2018).

Numerous strategies to reduce or remove invasive mongoose populations on islands, including trapping and toxic baiting, have been used over the years, mainly to reduce mongoose predation in and around sensitive native areas (e.g. ground nesting upland and sea bird colonies) (Barun et al. 2011; Sugihara et al. 2018; Berentsen et al. 2018). Trapping has been effective short-term at reducing predation risks in certain circumstances. However, trapping is labor-intensive, often expensive, only removes individuals from a limited area, and can ultimately prove ineffective due to the immigration of mongooses from outside the trapping areas (Hays and Conant 2007; Barun et al. 2011; Berentsen et al. 2018). As a result, toxic baiting has been

advocated as a way of increasing the probability of successfully controlling or eradicating mongooses (Barun et al. 2011; Sugihara et al. 2018). However, few toxicants have been developed or registered specifically for mongooses in the U.S. or elsewhere (Barun et al. 2011).

Here, we review the development and registration of mongoose toxicants in the U.S. to date. We then present a registration and use feasibility assessment comparing four of the most promising toxicants from a previous laboratory efficacy trial. The primary constraint considered in this assessment was cost, given that there has been little commercial interest in developing a toxic bait for mongoose in the U.S., so funding would be limited to public sources. Other constraints considered in the assessment were delays to registration, humaneness, antidote availability, and convenience-of-use of the toxicant. The purpose of this feasibility assessment is to help future research efforts select one or more of these toxicants for further development into an alternative toxic bait for mongooses with higher efficacy than what is currently available.

## Background

### *Registered toxicants for mongooses*

In the U.S., toxic baits for mongooses must be registered at the federal level by the U.S. Environmental Protection Agency (USEPA) as a pesticide under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA; Public Law No. 61-152, 7 U.S.C. § 136) and then also by individual states and territories under their governing pesticide laws before they can be distributed and used. Within the limitations set forth by FIFRA Section 24(c), states can also register pesticides for distribution and use only within their state for pest problems that are local in nature and for which an appropriate federal registration is not already available (40 C.F.R. § 162). Registrations under Section 24(c) are also called Special Local Needs (SLN) registrations. Most of the time, SLN pesticides are identical in composition or formulation to a “parent” federal registration, but the SLN pesticide label allows for additional uses in that particular state than what might be allowed on the parent label.

To date, the only pesticides registered specifically for mongooses in the U.S. were three SLN pesticides registered in Hawaii in the 1990s. The active ingredient in all of these SLN registrations was diphacinone (CAS No. 82-66-6), which is a “first generation” anticoagulant primarily used for rodenticide baits and that typically requires multiple successive feedings to be lethal (USEPA 2015). Under FIFRA, a pesticide active ingredient is “...an ingredient which will prevent, destroy, repel, or mitigate any pest” (40 C.F.R. § 136(a)(1)). Diphacinone is highly toxic to mongooses with a median lethal dose (LD<sub>50</sub>) of 0.18 mg per kg body weight (Keith and Hirata 1988a; Keith et al. 1989).



Studies conducted in the 1980s showed that diphacinone mixed into fresh meat baits was highly effective for mongooses (Keith et al. 1988; Keith and Hirata 1988b). Thus, the first SLN registration in Hawaii in 1991 was for a product named “Diphacinone Concentrate” (SLN Reg. No. HI-910004, EPA Reg. No. 12455-9), which was mixed by applicators into raw ground beef for a final concentration of 0.00025% diphacinone. This concentration was 20 times lower than the 0.005% diphacinone concentrations in rodenticide baits commercially available today (USEPA 2015). Although these fresh baits were efficacious, they were labor intensive to use and degraded quickly in the field (Sugihara et al. 2018). The SLN registration was eventually discontinued due to limited use.

The second SLN registration was a hard, waxy, grain-based, fish-flavored, rodenticide bait block named “Eaton’s® All Weather Bait Blocks® Rodenticide with Fish Flavorizer™” (0.005% diphacinone), first registered in 1997 for mongooses and rodents in Hawaii (SLN Reg. No. HI-970007, EPA Reg. No. 56-44). This bait appeared to have high efficacy for mongooses in two small-scale field applications on Oahu, Hawaii in 1998 (Smith et al. 2000). This SLN registration was eventually discontinued for unknown reasons, but it may also have been due to issues with bait longevity in the field and concerns about viable exotic plant seeds within the bait matrix (R. Sugihara, *pers. comm.*). The manufacturer also cancelled the parent Section 3 registration in October 2004.

The third SLN registration for mongooses in Hawaii is also a hard, waxy, grain-based, fish-flavored bait block (0.005% diphacinone) named “Ramik® Mini Bars Kills Rats and Mice”, which was first registered in 1998 (SLN No. HI-980005, EPA Reg. No. 61282-26). This SLN registration was still registered in Hawaii for use on both mongooses and rodents through 2018, and is being considered for renewal. Its use is restricted to bait stations in conservation areas with prior approval from the U.S. Fish and Wildlife Service. Bait stations are enclosed application devices that allow target species to access the bait, but prevent or limit access to humans and non-target species. This SLN registration is further classified as a restricted use pesticide (RUP; 7 U.S.C. § 136(d)). RUPs must be purchased and applied by certified applicators (40 C.F.R. § 136(e)), who are typically certified by the state in which the pesticide will be applied. However, this registered bait block had fairly low efficacy (20% mortality;  $n = 10$ ) over a 5-day feeding period in a laboratory no-choice efficacy trial using wild-caught mongooses from Hawaii, which was likely due to low palatability and consumption of the bait rather than low toxicity to mongooses (Sugihara et al. 2018). This product remains the only registered toxicant available for mongoose control in the U.S.

#### *Candidate toxicants for future research and development efforts*

Additional research is needed to develop a toxic bait that is more effective for controlling mongooses in the U.S., but that also has acceptable non-

target risks, and is not prohibitively costly or time consuming to develop and register. An ideal toxic bait would also have a humane mechanism of action, an antidote for accidental poisonings, and be convenient for applicators to store and use. However, not all of these goals may be obtainable for the active ingredients available for mammal pests at this time.

In no-choice efficacy trials for mongooses, Sugihara et al. (2018) evaluate the efficacy of nine active ingredients available in commercial rodenticide baits registered in Hawaii and/or had been previously tested for mongoose or used for other mammalian pests in Australia and New Zealand. They identified the four active ingredients, out of nine tested, with the most potential for use in toxic baits for mongooses. The active ingredients bromethalin, diphacinone, and para-aminopropiophenone (PAPP) appeared to be the most efficacious for mongooses. Although sodium nitrite (SN) showed relatively low efficacy, SN was also considered to be promising if used in a different bait formulation due to its possessing other favorable characteristics relative to many of the other active ingredients tested.

Bromethalin (CAS No. 63333-35-7) is an acute neurotoxin that requires only a single feeding to result in mortality. Bromethalin is a non-anticoagulant active ingredient used in a number of rodenticide baits registered for rodents in the U.S. (USEPA 2016a). Sugihara et al. (2018) tested a hard, waxy bait block (0.01% bromethalin) that is commercially available for use in bait stations to control rats and mice (Tomcat® Brands, Motomco). This bait had an efficacy of 95% mortality ( $n = 20$ ). The Tomcat bait block appeared to be relatively palatable to mongooses (average daily consumption was  $\sim 19\%$  of the bait offered; Sugihara et al. 2018) despite that bromethalin suppresses appetite once a lethal dose has been ingested (Jackson et al. 1982). To our knowledge, bromethalin has not been tested in mongooses in any other bait formulations.

Diphacinone is found in a number of rodenticide baits registered in the U.S. (USEPA 2015). Diphacinone is the most studied active ingredient to date for mongooses, in part because it appears to be particularly toxic to them and causes no taste aversion (Keith et al. 1989; Smith et al. 2000; Sugihara et al. 2018). Sugihara et al. (2018) found that diphacinone mixed with minced chicken (0.005% diphacinone) was highly palatable to mongooses with 100% daily consumption of the bait offered ( $n = 20$ ). The overall mortality rate was 70% for mongooses after a single day of feeding ( $n = 10$ ), and 100% for mongooses over a 3-day feeding period ( $n = 10$ ). Two commercially-available 0.005% diphacinone rodenticide baits from the Ramik rodenticide product line were also tested in this study over a 5-day feeding period: 1) the mini bar bait block currently registered in Hawaii for mongooses (described above), and 2) a hard, waxy pellet bait, which is only registered in Hawaii for rodents. These two diphacinone baits had much lower efficacy than the diphacinone mixed with minced chicken, likely due

to the much lower average daily consumption rates. Both of these baits are fish-flavored, grain-based, mold- and moisture-resistant baits optimized for gnawing rodents (Neogen Corporation 2012).

Unlike the first two active ingredients, PAPP (CAS No. 70-69-9) is not contained within any registered pesticides in the U.S., but is found in toxic baits registered for red foxes (*Vulpes vulpes* Linnaeus, 1758) and dingoes or wild dogs (*Canis lupus dingo* Meyer, 1973) in Australia (APVMA 2015), and for stoats (*Mustela ermine* Linnaeus, 1758) and feral cats (*Felis catus* Linnaeus, 1758) in New Zealand (ERMA 2011a; Eason et al. 2014). PAPP was also effective in canid ejector devices for red foxes and wild dogs in Australia (Allen 2019). PAPP is an acute, single-feeding toxicant that causes fatal methemoglobinemia at sufficient doses. PAPP is highly reactive and must be microencapsulated prior to mixing within a bait matrix in order to prevent taste aversion and chemical decomposition. Sugihara et al. (2018) tested three different microencapsulated PAPP (mePAPP) products mixed with minced raw chicken and found 0.15% PAPP to have the best efficacy (100% mortality; n = 10 animals) after a single feeding, with mongooses consuming about 60% of the bait offered on average.

Finally, SN (CAS No. 7632-00-0) is also not an active ingredient in any registered pesticides in the U.S., but an SN bait for feral swine (*Sus scrofa* Linnaeus, 1758) is being tested under an Experimental Use Permit (EUP; EPA Reg. No. 56228-EUP-42) in Texas and Alabama by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS). USDA APHIS and collaborators have generated or contracted all of the registration data required for SN as part of the development of a toxic bait for feral swine. SN baits are currently registered for common brushtail possums (*Trichosurus vulpecula* Kerr, 1792) and feral swine in New Zealand (NZEPA 2013) and are being reviewed for registration for feral swine in Australia (Linton Staples, *pers. comm.*). SN is also being tested for possible use in ejector devices for wild dogs and red foxes in Australia (Benjamin Allen, *pers. comm.*). Similar to PAPP, SN is an acute, single-feeding toxicant via fatal methemoglobinemia, but requires that a lethal dose is consumed over a relatively short period of time (i.e. a single feeding event or multiple feedings close together) because it is rapidly metabolized by the target animal (Lapidge and Eason 2010). Also like PAPP, SN is microencapsulated when used in baits to prevent taste aversion and degradation prior to consumption. SN rapidly dissociates to sodium and nitrite ions in the presence of moisture or acids within a bait matrix or the target animal. Microencapsulation of the SN masks the overly salty flavor and other aversive tastes or smells that result from the decomposition of nitrite into nitric oxides, which can slow or inhibit consumption by the target animal.

In Sugihara et al. (2018), two SN bait prototypes contained microencapsulated SN (meSN) mixed with minced raw chicken at a 5% SN concentration. Both baits had relatively poor efficacy (10 and 30% mortality,  $n = 10$  per bait), which was likely due to insufficient bait consumption and sublethal dosing. Desiccation of the minced raw chicken occurred within a few hours after it was mixed with meSN, which is consistent with changes that would occur if the microencapsulation on the SN had been compromised. The proprietary microencapsulation formula was likely water soluble (Linton Staples and Duncan McMorran, *pers. comm.*). Thus, the SN would have become detectable to mongooses by taste, which likely reduced and slowed consumption of the baits during the 1-day feeding trial, limiting their efficacy. In prior studies on feral swine and common brushtail possums, SN that was not microencapsulated before it was mixed into a bait matrix has caused similar taste aversion, which resulted in low bait consumption and low to zero efficacy (Cowled et al. 2008; Foster et al. 2014; Shapiro et al. 2016).

An alternate bait matrix that better preserves the microencapsulation on the SN (e.g. an oil-based or dry matrix) might prevent taste aversion and improve efficacy. For example, a pen efficacy study of a bait for feral swine containing meSN (10% SN) within a peanut paste bait matrix resulted in 93% mortality after one night of feeding (Snow et al. 2017). Alternately, the use of a water-resistant microencapsulation material may also result in better efficacy in wetter bait matrices. The efficacy of SN at different concentrations and using a compatible microencapsulation formula and bait matrix has not yet been thoroughly tested for mongooses.

#### *U.S. pesticide registration requirements*

The USEPA must be provided with specific data from standardized product chemistry, ecological effects, toxicology, and environmental fate studies before they will consider registering any pesticide product (40 C.F.R. § 158). The proposed use pattern for an end-use product (EP; e.g., a toxic bait) also determines which set of registration data will be required (40 C.F.R. § 158.100). The majority of the data required for a registration application are for the technical grade of the active ingredient (40 C.F.R. § 158). A smaller subset of product chemistry, toxicology, and efficacy data are also required for registration of each new EP (e.g. a commercial “off-the-shelf” toxic bait or mix-on concentrate product). Additional data is required for the active ingredient when an EP registration application proposes a use pattern (e.g. a new use site, application method, or target species) that is not yet registered for that active ingredient. The studies that produce these data must usually conform strictly to USEPA’s study guidelines (40 C.F.R. § 158.70) and be performed in accordance with USEPA’s FIFRA Good Laboratory Practice (GLP) standards (40 C.F.R. § 160). Most of these studies can be contracted

from private laboratories that specialize in conducting guideline studies for pesticide registration. Individual study costs can range from a few hundred dollars to over a million dollars. Alternatively, applicants can choose to submit a data waiver request in which they provide justification for why a particular data submission is not necessary, applicable to the active ingredient or EP, or to the proposed application methods or use pattern for the EP (40 C.F.R. § 158.45). When USEPA reviews a registration application, they will accept or reject any data submission or waiver request. They may also require additional data on a case-by-case basis (40 C.F.R. § 158.75). All of this makes predicting the total registration data costs for a mongoose toxicant difficult.

A mongoose EP would be classified as having a terrestrial outdoor (non-food) use pattern. Some of the data for this use pattern might be waived by USEPA if the likely risks of the proposed use pattern (e.g. bait station only) and/or toxicity of the active ingredient and/or EP are low. Conversely, additional data might be required by USEPA if the product or use pattern exhibits high risk characteristics for human health, non-target species, or the environment. For mongoose EPs containing a registered active ingredient (i.e. an active ingredient that is already contained in a Section 3 registered EP), many if not all of the data requirements would have already been satisfied or waived for the active ingredient, and could be cited with the permission of the data owners. This also holds true for data on an EP if the EP is already registered for other target species.

The proposed application methods and/or toxicity of the active ingredient and EP and risks to non-target species also determine whether or not the EP will be classified as an RUP (40 C.F.R. § 152.170). Even EPs allowed for general use can have limitations on the label as to who can purchase and how they are allowed to use them. Some proposed application methods may never be registered if USEPA determines the risks to outweigh the benefits, or they may be limited to a small group of users under specific circumstances.

The general categories of data required for an EUP application for a field efficacy trial and then a Section 3 registration application for any mongoose EP are summarized below and are detailed in 40 C.F.R. § 158.

#### Laboratory and field efficacy data

EPs used to control vertebrates that may directly or indirectly transmit diseases to humans must provide product performance (efficacy) data for the EP for the target species, typically from both laboratory and field efficacy studies.

#### Product chemistry data

The product chemistry data requirements are fairly standardized for any unregistered active ingredient or EP. The “Group A” data requirements

describe the EP's composition (identify the active and inert ingredients), the production process for the active ingredient, the formulation process for the EP, and the formation of any impurities during the production or formulation process. The Group A data submission must also demonstrate the consistency of the EP and provide an enforcement analytical method for testing the EP for the concentration of the active ingredient and any impurities of toxicological concern. The "Group B" data requirements include the determination and description of a wide range of physical and chemical properties of the active ingredient and the EP, such as color, pH, vapor pressure, storage stability, etc.

#### Toxicology data

The toxicology data requirements for an active ingredient used in an EP with a terrestrial outdoor and non-food use pattern include a number of acute toxicity, subchronic toxicity, chronic toxicity, genetic toxicity, neurotoxicity, immunotoxicity, and other special human health effects studies. A standard suite of six acute toxicity studies ("the six-pack") and a subchronic dermal toxicity study are also required for each non-food use EP. These data are used by USEPA to assess hazards to humans and domestic animals that could potentially be exposed to the active ingredient through use of the EP.

#### Ecological effects (non-target risks) data

Ecological effects data requirements for a terrestrial outdoor use pattern include studies looking at the acute and chronic toxicity of the active ingredient to a variety of non-target bird, mammal, fish, and terrestrial and aquatic invertebrate species, and sometimes plants. These data are then used to assess primary and secondary risks to non-target species, including endangered species.

Primary risks are the risks to target or non-target animals that consume the EP or to non-target animals or plants that come into direct contact with the EP. Some EPs can cause emesis in animals, resulting in partially digested toxic bait on the ground. Primary risks are determined by the toxicity of the active ingredient to non-targets and on the amounts and routes of direct exposure non-targets could have to the active ingredient in the EP.

Secondary risks are risks to predatory or scavenging animals that feed on target or non-target animals that fed on toxic bait. Many active ingredients result in toxic tissue residues, which can then be consumed by predators or scavengers. Additionally, some active ingredients have the potential for bioaccumulation up the food chain.

#### Environmental fate data

The environmental fate data requirements are usually required for just the active ingredient. These data requirements include studies on the hydrolysis,



photodegradation, and soil and aquatic metabolism of the active ingredient, and the leaching and adsorption or desorption properties of the active ingredient in soils. These data are used to assess the distribution and persistence of the active ingredient and any degradation products in the environment.

### **Feasibility assessment**

Following from Sugihara et al. (2018), we conducted a product feasibility assessment on theoretical EPs for mongooses containing bromethalin, diphacinone, PAPP, or SN, assuming that a sufficiently attractive and thus, efficacious EP could be developed for each one. The feasibility assessment included the predicted cost and time to register with USEPA and potential factors affecting operational use, such as relative humaneness, availability of an antidote, and overall convenience of use.

#### *Cost and time feasibility*

In order to compare the likely cost of registering an EP for mongooses containing one of these four active ingredients, we compiled the set of supporting data that would likely be required by USEPA for each EP under two registration scenarios that differed by which bait application methods would be allowed on the pesticide label. We focused on the data that would be required for a federal (Section 3) registration rather than a state-limited SLN, because mongooses are invasive to U.S. territories in addition to Hawaii. Furthermore, SLN registrations are only allowed for active ingredients (and inert or other ingredients) already found in a federally-registered pesticide (40 C.F.R. § 162.152), and two of the active ingredients reviewed here were not.

We determined the sets of studies still needed for each active ingredient for a range of scenarios by 1) using the registration data requirements outlined in 40 C.F.R. § 158, 2) reviewing what data are already available for each active ingredient and the data gaps identified by USEPA for bromethalin and diphacinone in recent registration reviews (USEPA 2015, 2016a), and 3) comparing to the data sets USEPA has required for rodenticides and other vertebrate pesticides with similar application methods (USEPA 2008, 2016b, 2018). For one set of scenarios, the label for the EPs would only allow two of the most conservative application methods for vertebrate pesticides, which are bait station and burrow baiting applications. USEPA generally considers these application methods to be the lowest risk for applicators, non-target species, and the environment (discussed in more detail below), and typically require smaller sets of supporting data (e.g. see USEPA 2016b). In the other set of scenarios, the data sets included the additional data that would likely be required if the labels allowed aboveground spot baiting and hand broadcast (thrown bait) applications in

addition to bait stations and burrow baiting. Aboveground applications outside of bait stations are typically considered higher risk (discussed below) and usually require more registration data to support these uses.

For any data on the active ingredient that was already accepted by EPA in support of existing diphacinone or bromethalin EPs, we assumed that the study cost would be zero, because the original data submitter would agree to share the data at no cost or the data would be old enough (> 15 years) that the original data rights had expired (40 C.F.R. § 152.93(b)(3)). For any remaining data requirements that were never submitted to USEPA, but would likely be required for the bait application method scenario, we estimated the study costs based on quotes obtained from private U.S. contract laboratories for 2018. Note that these study costs will gradually increase over time due to inflation and other market factors.

Because EPA could agree to waive some of the required data for a particular EP or active ingredient, we also provided a range of data costs for a least expensive (“best case”) and a most expensive (“worst case”) registration scenario (discussed in more detail below). Note that for any of the active ingredients, USEPA may require additional non-guideline ecological effects studies for an unregistered EP that is not similar to commercially-available rodenticide formulations (e.g. a new meat-based bait EP) to determine whether or not non-target wildlife or terrestrial invertebrates are at acute or chronic risk from the novel bait formulation, carcasses, or vomitus (if applicable). Because these studies are often only conditionally required or are non-guideline (i.e. not standardized), and customized for the specific active ingredient, it was not possible to estimate these potential additional study costs for this review.

USEPA has different statutorily-determined decision times (review periods) for EUP and Section 3 registration applications for registered and unregistered active ingredients and for amended or new uses under the Pesticide Registration Improvement Extension Act of 2018 (PRIA 4; P.L. 116-8). We determined the relevant decision times for each active ingredient and EP option using EPA’s online PRIA 4 Determination Decision Tree (USEPA 2019).

### Bait station and burrow baiting applications

The use of enclosed bait stations for any aboveground applications can significantly reduce the risks to non-target animals, given that most cannot access the bait stations. “Tamper-resistant” enclosed bait stations are commonly required by USEPA for use of rodenticide EPs aboveground. However, large or strong animals may still be able to access these bait stations. Feral swine have been documented destroying plastic bait stations used in a conservation rodent control efforts in Hawaii, and consuming the diphacinone baits they contained (Pitt et al. 2005). However, for the sake of



this review, bait stations were presumed to be constructed of materials resistant to large animals when they are used in areas where these non-target species occur. Applications made only within the openings of burrows (burrow baiting) also reduce the risk to non-target species that cannot access the burrows. Because these application methods limit exposure of non-target animals, the registration data requirements for these application methods are typically fewer than for application methods that have greater risk of exposure.

Bromethalin and diphacinone already have EPs registered by USEPA for use in bait stations and below ground hand applications in burrows. The data requirements for these rodenticides based on these registered use patterns was recently reevaluated by USEPA's Hazard and Science Policy Council (USEPA 2016b) and during recent registration reviews by USEPA for both chemicals (USEPA 2015, 2016a). For an already-registered bromethalin or diphacinone EP under the best case scenario, new registration data would likely be limited to laboratory and field efficacy data on the EP for mongooses. Under the worst case scenario, a few additional data requirements for the active ingredient would be required in addition to the efficacy data on the EP based on what has not yet been submitted to or accepted by USEPA to date (USEPA 2015, 2016a).

For an unregistered bromethalin or diphacinone EP under the best case scenario, new registration data would include the laboratory and field efficacy data plus the standard product chemistry and acute toxicity data that are required for any new EP (assuming USEPA did not allow "bridging" or the use of data from similar EPs). Again, the worst case registration data cost estimates include the same few additional data requirements for the active ingredient based on what has not yet been submitted to or accepted by USEPA to date (USEPA 2015, 2016a).

However, it is not anticipated that any additional data on these active ingredients would be required for a mongoose EP with bait station or burrow baiting application methods. Therefore, the data costs are the lowest and USEPA review times are the shortest for EPs containing bromethalin or diphacinone when used in bait stations and burrow baiting applications only, and particularly for already registered EPs (Table 1).

Although SN is not a registered active ingredient (i.e., there are no registered EPs) with USEPA at this time, all of the registration data required by USEPA for SN for an EP used for bait station applications has already been submitted to USEPA or contracted by USDA APHIS as part of development of an SN EP for feral swine. Furthermore, given that nitrite is a component of the nitrogen cycle, and much is already known about the fate of nitrite in terrestrial and aquatic ecosystems, it is not anticipated that any additional environmental fate data would be required for an EP with a burrow baiting application method. Therefore, an unregistered SN EP for use in bait stations and burrow baiting applications would be similar in registration

**Table 1.** Total estimated registration data costs and EPA decision times (review periods) for end-use products (EPs; toxic baits) containing bromethalin, diphacinone, para-aminopropiophenone (PAPP), or sodium nitrite (SN) for use in bait station or burrow baiting applications only. Total registration data cost estimates include the data required for both the experimental use permit (EUP) and subsequent Section 3 registration applications. Best case scenarios assume USEPA will waive some data requirements as discussed under “Bait station and burrow baiting applications.” Worst case scenarios assume USEPA will not waive these data requirements.

Active ingredient	Registered or Unregistered EP	Total registration data cost scenarios <sup>a</sup>		Decision time <sup>b</sup> (months)	
		Best case	Worst case	EUP	Section 3
Bromethalin	Registered	\$125,000	\$200,000	6	4–10
	Unregistered	\$220,000	\$300,000	6	10–12
Diphacinone	Registered	\$125,000	\$200,000	6	4–10
	Unregistered	\$220,000	\$300,000	6	10–12
PAPP	Unregistered	\$810,000	\$5,800,000	16	21
SN	Unregistered	\$220,000	\$300,000	16	21

<sup>a</sup>Registration data cost estimates were summed from study quotes obtained from contract laboratories in 2018, and do not include initial research and development or pilot study costs on the EP.

<sup>b</sup>USEPA’s statutorily-determined decision times for different types of registration applications are specified under the Pesticide Registration Improvement Extension Act of 2018. Review periods begin once all the necessary data have been collected and the registration application is submitted to USEPA.

data costs to an unregistered bromethalin or diphacinone EP, but would have longer review times (Table 1) because USEPA has not yet reviewed and accepted data on the active ingredient.

In contrast to the other three active ingredients, a great deal of registration data is still missing for the unregistered active ingredient PAPP. Relatively little registration data that meets USEPA’s study guidelines or that was conducted under FIFRA GLPs or equivalent was available for PAPP from the registrations of PAPP products in Australia (APVMA 2015) or New Zealand (ERMA 2011b). The best case estimate of registration costs for PAPP assumed that USEPA would accept all of the data waiver requests that could conceivably be justified or studies in the published literature that are close but do not fully meet the USEPA’s guideline requirements. The worst case estimate for PAPP assumed that only the most suitable data available from the Australian or New Zealand registrations would be accepted by USEPA, and almost all of the other data requirements would require new GLP studies. Even under the best case scenario, the cost to register a PAPP EP used for bait stations or burrow baiting applications would likely be many times more expensive than the cost to register a bromethalin, diphacinone, or SN EP (Table 1). USEPA review times are also many months longer for a PAPP EP than for a bromethalin or diphacinone EP, but the same as for an SN EP (Table 1).

#### Aboveground spot baiting and hand broadcast applications

Primary risks to non-target animals are a major concern for a vertebrate EP applied aboveground and outside of a bait station, particularly for acute, single-feeding toxins when non-target animals could easily consume a lethal dose within the bait exposure period (USEPA 1998, 2008, 2016a). An EP that allowed aboveground spot baiting or hand broadcast applications would likely require additional ecological effects and environmental fate data

**Table 2.** Total estimated registration data costs and EPA decision times (review periods) for end-use products (EPs; toxic baits) containing bromethalin, diphacinone, para-aminopropiophenone (PAPP), or sodium nitrite (SN) for use aboveground spot baiting or hand-broadcast applications in addition to bait station and burrow baiting applications. Total registration data cost estimates include the data required for both the experimental use permit (EUP) and subsequent Section 3 registration applications. Total estimated data costs include those listed Table 1. Best case scenarios assume USEPA will waive some data requirements as discussed under “Aboveground spot baiting and hand broadcast applications”. Worst case scenarios assume USEPA will not waive these data requirements.

Active ingredient	Registered or Unregistered EP	Total registration data cost scenarios <sup>a</sup>		Decision time <sup>b</sup> (months)	
		Best case	Worst case	EUP	Section 3
Bromethalin	Registered	\$172,000	\$430,000	6	15
	Unregistered	\$267,000	\$530,000	6	15
Diphacinone	Registered	\$125,000	\$200,000	6	9–15
	Unregistered	\$220,000	\$300,000	6	10–15
PAPP	Unregistered	\$1,040,000	\$6,750,000	16	21
SN	Unregistered	\$275,000	\$740,000	16	21

<sup>a</sup>Registration data cost estimates were summed from study quotes obtained from contract laboratories in 2018, and do not include initial research and development or pilot study costs on the EP.

<sup>b</sup>USEPA’s statutorily-determined decision times for different types of registration applications are specified under the Pesticide Registration Improvement Extension Act of 2018. Review periods begin once all the necessary data have been collected and the registration application is submitted to USEPA.

compared to bait station or burrow baiting uses. These data are typically required for the active ingredient rather than for the EP, and are used by USEPA in standardized risk models.

Bromethalin, SN, and PAPP are acute, single-feeding toxicants that do not currently have registered EPs that allow aboveground baiting outside of bait stations or are unregistered active ingredients with USEPA. Under the best case scenario for an EP containing the registered active ingredient bromethalin, additional registration data required by USEPA would likely include a subset of the unfilled ecological effects or environmental fate data on the active ingredient (for data gaps, see USEPA 2016a). Under the worst case scenario, data required would include almost all of the unfilled ecological effects or environmental fate data on the active ingredient. The difference between the best case and worst case scenarios for the unregistered active ingredient SN and PAPP is how many data waiver requests would be accepted for the full set of ecological effects and environmental fate data requirements on the active ingredient.

Under these scenarios, EPs containing bromethalin, SN, or PAPP would likely be the most expensive of the four active ingredients to register for mongooses for aboveground spot baiting or broadcast application methods, with PAPP being the most expensive of the three (Table 2). Even with submission of all required data, USEPA would still likely limit broadcast applications of an EP containing an acute, single-feeding toxicant to areas where non-target animals could be excluded or were unlikely to be exposed to or at primary risk from the bait itself (e.g. see the USEPA (2008) risk assessment for rodenticides).

In contrast, the primary risks from aboveground spot or broadcast baiting are often reduced for active ingredients that require multiple feedings to achieve toxicity and that have relatively short persistence of

residues in tissues (USEPA 1998, 2015). Diphacinone is the only active ingredient of the four reviewed here that requires multiple feedings to be toxic, lowering primary risks, and the secondary risks for diphacinone are lower than for other commonly used rodenticide anticoagulants (Fisher et al. 2003; McLeod and Saunders 2013; USEPA 2015). However, diphacinone likely poses higher secondary risks to non-target species, particularly scavengers, than the other three active ingredients evaluated here (Eason et al. 2014; USEPA 2016a; Shapiro et al. 2018).

USEPA currently allows aboveground spot baiting and hand broadcast uses for a number of commercially-available diphacinone rodenticide baits, and aerial broadcast uses in conservation areas (e.g. Diphacinone-50: Pelleted Rodenticide Bait for Conservation Purposes; USEPA Reg. No. 56228-35; USEPA 2015). Therefore, it is likely that all of the required registration data for diphacinone for these types of application methods has been submitted to or waived by USEPA, and no additional registration data would be required for an EP containing diphacinone, assuming the concentration of diphacinone was at or below the concentration in currently registered EPs (Table 2). Because of this, the EPA review times under PRIA 4 would also be the shortest for diphacinone.

### *Operational feasibility*

#### Humaneness

Under FIFRA, the humaneness of a toxicant's mechanism of action is not considered during the EPA's review for registration of a pesticide in the U.S. However, if an EP is not perceived to be humane, the extent that it is used on the ground could be limited by lack of support from stakeholders and potential users, and by lack of public acceptance of control efforts, particularly when the target species is a mammal.

We compared the relative humaneness of the four active ingredients using several metrics commonly evaluated for toxicants, including level of awareness after onset of symptoms, clinical signs of distress or observable symptoms prior to death, severity of symptoms, duration of symptoms (time period when first symptoms appear until death), and time to death. These data were compiled from the literature for mongooses (Sugihara et al. 2018) and a representative group of other mammalian species (Table 3; Jackson et al. 1982; Savarie et al. 1983; Dreikorn and O'Doherty 1984; Dorman et al. 1990; Marks et al. 2004; Eason et al. 2010; IMVS 2010; Landcare Research 2010; Foster 2011; McLeod and Saunders 2013; USEPA 2015, 2016a; Shapiro et al. 2016; Snow et al. 2017; Allen 2019). In order to compare the four active ingredients, we gave each a rank order from 1 (most humane) to 4 (least humane) for each humaneness metric (Table 4). When it was unclear which of two active ingredients ranked higher or lower (e.g. it was difficult to determine whether the symptoms of the first

**Table 3.** Humaneness metrics evaluated for each active ingredient from data compiled from the literature for a range of carnivorous and omnivorous mammalian species.

	Active ingredient			
	Bromethalin	Diphacinone	Para-aminopropiophenone (PAPP)	Sodium nitrite (SN)
<u>Humaneness metric</u>				
Mode of action	Neurotoxin	Anticoagulation	Methemoglobinemia	Methemoglobinemia
Level of awareness after onset of symptoms	Not reported, assumed conscious until death	Conscious until death	Loss of responsiveness occurs with increase in symptoms Loss of consciousness occurs prior to death	Loss of responsiveness occurs with increase in symptoms Loss of consciousness occurs prior to death
Clinical signs of distress or observable symptoms prior to death	Salivation Hyperactivity Hyperesthesia Myoclonia Vocalization Lethargy Hind-leg weakness Tremors Lateral recumbence Convulsions Seizures Paralysis Semicoma	Internal hemorrhage External hemorrhage Anorexia Dyspnoea Hypersensitivity Tremors Emesis Abnormal movement Lateral recumbence	Lethargy/weakness Salivation Nausea Emesis Hyperventilation Dyspnoea Cyanosis Vocalization Lateral recumbence Paddling/writhing Seizures	Lethargy/weakness Salivation Nausea Emesis Breathlessness Dyspnoea Pale skin Cyanosis Tremors Incoordination Lateral recumbence Paddling/writhing Seizures
Severity of symptoms	Severe to extreme	Severe to extreme	Mild to extreme	Mild to extreme
Duration of symptoms (period from first symptoms to death)	< 1–3 days	1–2 days to weeks	Minutes to hours	Minutes to hours
Time to death	< 1–4 days	3–21 days	< 1 hour–< 1 day	< 1 hour–< 2 days
<u>Species represented<sup>a</sup></u>	Domestic cat Domestic dog House mouse Mongoose Norway rat	Ferret House mouse Mongoose Norway rat	Coyote Domestic cat Domestic dog Ferret Mongoose Red fox Stoat Wild dog	Common brushtail possum Feral swine Mongoose Raccoon

<sup>a</sup>Scientific names: common brushtail possum (*Trichosurus vulpecula* Kerr, 1792); coyote (*Canis latrans* Say, 1823); domestic cat (*Felis catus* Linnaeus, 1758); domestic dog (*Canis lupus familiaris* Linnaeus, 1758); feral swine (*Sus scrofa* Linnaeus, 1758); ferret (*Mustela putorius furo* Linnaeus, 1758); house mouse (*Mus musculus* Linnaeus, 1758); mongoose (*Herpestes javanicus auro-punctatus* Hodgson, 1836); Norway rat (*Rattus norvegicus* Berkenhout, 1769); raccoon (*Procyon lotor* Linnaeus, 1758); red fox (*Vulpes vulpes* Linnaeus, 1758); stoat (*Mustela ermine* Linnaeus, 1758); wild dog *Canis lupus dingo* Meyer, 1973).

active ingredient were more severe or caused more distress than the symptoms of the second active ingredient), both active ingredients were given the average of the two ranks they would have held. Overall humaneness was then compared across active ingredients based on the summed rank score for the five metrics.

Diphacinone ranked the least humane overall, primarily due to the longer duration of symptoms and time to death compared to bromethalin, which was ranked second to last. SN and PAPP were tied for most humane because their mode of action (fatal methemoglobinemia) generally causes

**Table 4.** Relative rank (1–4) of the four active ingredients for each humaneness metric and their overall humaneness rank (the sum total).

Humaneness metric	Relative rank <sup>a</sup>			
	Bromethalin	Diphacinone	Para-aminopropiophenone (PAPP)	Sodium nitrite (SN)
Level of awareness after onset of symptoms	3.5	3.5	1.5	1.5
Clinical signs of distress or observable symptoms	3.5	3.5	1.5	1.5
Severity of symptoms	3.5	3.5	1.5	1.5
Duration of symptoms (period from first symptoms to death)	3	4	1.5	1.5
Time to death	3	4	1.5	1.5
<b>Overall humaneness rank (sum total)</b>	<b>16.5</b>	<b>18.5</b>	<b>7.5</b>	<b>7.5</b>

<sup>a</sup>When two active ingredients tied in rank order or were difficult to rank (e.g. it was difficult to determine which symptoms were the most severe), we assigned the two active ingredients the average of the two ranks they would have held.

symptoms of lower severity and of shorter duration compared to the other two active ingredients, and because fatally dosed animals generally fall unconscious prior to the onset of the most severe symptoms.

#### Antidotes for accidental exposure

The four active ingredients also vary in the availability and efficacy of an antidote for humans or non-target animals in the event of an accidental poisoning, which might also affect public acceptance of a toxic bait, particularly one applied outside of bait stations or burrows. Bromethalin has no antidote in the event a toxic dose is ingested, but supportive therapies can limit or prevent toxicosis if administered quickly enough (Dorman et al. 1990; Coppock 2013; Rubinstein and Weinberg 2014). The antidote for diphacinone is vitamin K, which can be administered and still be effective for a longer period of time, largely because diphacinone is generally a slower acting toxicant that requires multiple feeding events (USEPA 2008, 2015; Baldwin et al. 2016). The antidote for a toxic dose of either PAPP or SN is methylene blue, which must be quickly administered intravenously due to the rapid onset and lethality of severe methemoglobinemia (NZEPA 2013; APVMA 2015; Shapiro et al. 2016).

#### Convenience of use

If an EP is not easy to use or store, toxic baiting efforts for mongooses are more likely to be inconsistently implemented or eventually abandoned. EPs that are classified as general use (unclassified) by USEPA are the easiest to purchase and use. EPs containing any of these four active ingredients could likely be classified as general use when only utilized within tamper-resistant bait stations and for burrow baiting by hand. However, these baiting application methods are more labor intensive than hand spot baiting and hand broadcast application methods.

Due to the primary risks of bromethalin, SN, and PAPP to most non-target vertebrate species, USEPA is unlikely to approve their widespread use aboveground and outside of bait stations (apart from rare circumstances



where non-target animals could be excluded or were not at risk from the EP itself). Diphacinone rodenticides are already registered for these application methods in conservation rodenticide baits and typically require multiple feedings for toxicity (USEPA 2015). Therefore, a diphacinone EP for mongooses could likely be registered for these application methods, given that the application rates would likely be lower than for rodents. However, like the diphacinone conservation rodenticide baits, any diphacinone EP for aboveground spot baiting or broadcast applications for mongooses would likely be classified as an RUP (at least for these application methods), due to the secondary risks to non-target animals (40 C.F.R. § 152.170(c); USEPA 2015). Furthermore, a diphacinone EP could still pose significant primary risks to non-target species if the bait palatability was universally high (e.g. Pitt et al. 2005). An RUP classification would make an EP less convenient to use compared to a general use EP, because applicators have to be certified by their state in the appropriate certification categories.

Furthermore, a mongoose EP must also be reasonably shelf-stable and resistant to degradation in hot or wet environments in order to be worth the effort from a manufacturing, distribution, marketing, or end-user standpoint. Although a fresh bait would likely be the most attractive to mongooses, it is highly perishable and logistically infeasible for larger scale applications, which is why the previous fresh diphacinone bait SLN registration was eventually abandoned (Pitt and Sugihara 2009; Barun et al. 2011; Sugihara et al. 2018). Longevity is particularly important for surveillance or rapid response scenarios where bait is likely to go unconsumed for long periods of time.

Thus, the ideal bait matrix from a palatability standpoint cannot outweigh other convenience-of-use factors and may not be necessary from an efficacy standpoint. A variety of bait flavors have been shown to be attractive to mongooses as they are opportunistic generalists (Pitt and Sugihara 2009; Berentsen et al. 2014, 2018; Pitt et al. 2015). Mold-resistant rodenticide EPs with a long shelf life have already been developed for bromethalin and diphacinone, and could potentially be modified to appeal more to mongooses while still retaining these characteristics. EPs with comparable stability have not yet been developed or registered in the U.S. for PAPP and SN. The fact that greater concentrations of PAPP and SN are required for toxicity for mongooses (Sugihara et al. 2018) and that they both require microencapsulation to mask their presence and slow their degradation also complicate EP development efforts for these two toxicants.

## Recommendations and discussion

Our feasibility assessment did not indicate a consistent winner among the four active ingredients when looking across all of the criteria or constraints

we considered. Therefore, because registration data costs are a hard constraint and will likely rely on limited public funds, and the need for an alternative toxicant is time sensitive, we prioritized registration data costs and decision times over other factors when making recommendations on further product development efforts. However, we further discuss the relative advantages or disadvantages of the other active ingredients in the event that an alternative active ingredient is needed for unforeseen reasons or to diversify the options available in the future.

Our feasibility assessment indicated that an EP containing diphacinone would be among the least expensive to register and has several additional advantages over a bait containing one of the other three active ingredients. First, because it is already a registered active ingredient for all of the application methods considered here, EPA's decision times would be among the shortest. Furthermore, because diphacinone is the only active ingredient of the four that usually requires multiple feedings for lethality (at least for most species) and there is an effective antidote, a diphacinone EP likely poses the lowest primary risk to non-target species (Baldwin et al. 2016). Although normally this characteristic might also be considered disadvantageous when used in bait stations compared to acute toxicants in terms of efficacy in the target species, diphacinone is particularly toxic to mongooses compared to other mammals, and often does not require a second or third feeding for lethality (Sugihara et al. 2018).

Any completely novel bait matrix for mongooses for any of the active ingredients would likely require a great deal of research and development work on the formulation before any of the registration data required for the EP can be completed. These development times and costs were not estimated in this review, but can be substantial. Therefore, an additional advantage that a diphacinone EP potentially has over a PAPP or SN bait, but perhaps not over a bromethalin bait, is that multiple shelf- and field-stable diphacinone rodenticide EPs are already registered in the U.S. and manufactured commercially. One of these EPs could potentially be more palatable and have higher efficacy than the SLN diphacinone bait currently registered in Hawaii for mongooses. Given that mongooses are particularly sensitive to diphacinone, an EP with increased palatability and higher bait consumption rates may not require several days of feeding, and shortened exposure periods could further reduce non-target risks.

A diphacinone EP did have some disadvantages in our feasibility assessment compared to the others when applied in bait stations and in burrows. When used in bait stations and for burrow baiting, the other three active ingredients would likely pose much lower secondary risks to non-targets consuming tissue residues of animals that had consumed the bait compared to a diphacinone EP (ERMA 2011b; Shapiro et al. 2018; USEPA 2008, 2015, 2016a). In addition, diphacinone was ranked the least humane overall of the four active ingredients in our humaneness assessment,



which could hinder future use in the field due to public opposition. However, given that three diphacinone SLN products have been registered and used to control mongooses in Hawaii to date and mongooses remain a priority species for removal, there is no indication that public resistance will be an issue for a future diphacinone EP for mongooses.

If an alternative active ingredient is needed for use in bait stations or burrow baiting applications, our feasibility assessment indicated that a bromethalin EP would be more humane than a diphacinone EP and would be cheaper and faster to register than a PAPP or SN EP. Further investigation and testing of existing bromethalin EPs is advised if developers have very limited funds for registration and need the product available quickly. However, we ranked bromethalin as less humane than SN and PAPP, and bromethalin does not have an antidote.

A PAPP or SN EP for use in bait stations and burrow baiting applications had some advantages relative to a diphacinone or bromethalin EP, if sufficient resources were available for registration. Of the four active ingredients, we ranked PAPP as one of the most humane for mongooses. There are also PAPP EPs that are already developed for carnivores and commercially available in Australia that might prove efficacious for mongooses as well. However, we estimated that a PAPP EP would be many times more expensive and one of the slowest to register relative to the other active ingredients largely because PAPP is an unregistered active ingredient and a lot of the registration data that would be required in the U.S. are lacking. Development of a PAPP EP for mongoose control will likely only be feasible in the U.S. if the registration data are generated for another target species with a larger commercial market. In contrast, an SN EP would be relatively inexpensive to register, but one of the slowest as an unregistered active ingredient. We also ranked SN as one of the most humane toxicants for mongooses. However, substantial additional research (pilot studies) and development efforts may be required to make an SN EP sufficiently shelf-stable and palatable for mongooses.

For any use pattern aboveground and outside of bait stations, such as spot baiting or hand broadcast application methods, a diphacinone EP has far and away the best chance for registration, and would be the least expensive and fastest to register of the four. Low primary risk to non-target species is critical for registration of an EP aboveground and outside of bait stations in places where vulnerable non-target species are present, which includes most of the places where toxic baiting for mongooses would be needed. A number of diphacinone EPs for rodents are already registered for broadcast uses in a variety of non-crop use sites in the U.S., including conservation areas. However, it should be noted that these types of application methods would almost certainly result in an RUP classification and require certified applicators, regardless of which active ingredient the EP contained.

Future testing and development efforts in the U.S. can use this assessment to develop an alternative EP for mongooses using one of these four active ingredients, or to utilize a similar approach to identify and compare the registration and use constraints of alternative active ingredients, if needed. The intended use patterns, including an evaluation of the relative merits of the application method such as bait station versus broadcast delivery, could also influence the selection of an active ingredient. Although our discussion is specific to the registration process in the U.S., other countries where mongooses are invasive usually have similar constraints and requirements (e.g. Costa Rica, Croatia, Japan, and Netherland Antilles), making consideration of our assessment worthwhile in an international context. Finally, despite being specific to selection of a toxicant for mongooses, this review may serve as a useful primer and template for managers considering development of toxicant products for other vertebrate pest species.

### Acronyms and abbreviations

CAS	Chemical Abstracts Service
C.F.R.	Code of Federal Regulations
EP	End-use product
EUP	Experimental Use Permit
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practice
mePAPP	microencapsulated para-aminopropiophenone
meSN	microencapsulated sodium nitrite
PAPP	Para-aminopropiophenone
PRIA 4	Pesticide Registration Improvement Extension Act of 2018
RUP	Restricted use pesticide
SLN	Special Local Need
SN	Sodium nitrite
U.S.C.	United States Code
USDA APHIS	U.S. Department of Agriculture, Animal and Plant Health Inspection Service
USEPA	U.S. Environmental Protection Agency

### Acknowledgements

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### Conflicts of interest

The authors declare no conflicts of interest.

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## **Appendix C: QA-2834 Study Protocol**

*This draft protocol is in preparation for submission to the NWRC Quality Assurance Unit and Institutional Animal Care and Use Committee. U.S. Fish and Wildlife Service concurrence on Endangered Species Act compliance is currently pending.*



**Study Title:** Two-choice laboratory efficacy test in mongooses - fish-based bait for mongooses (0.005% diphacinone)

**NWRC Study Director:** Robert Sugihara

**Approved NWRC Project:** Methods and strategies to manage invasive species impacts to agriculture, natural resources, and human health and safety

**SIGNATURE**

**DATE**

<b>Protocol approval by the Sponsor's Representative:</b>		
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**SIGNATURE**

**DATE**

<b>NWRC Study Director:</b>		
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Study Director's position (check one):

- Project Leader
- Research Scientist (non-project leader)
- Biologist/Chemist/Technician
- Student: NWRC Representative/Contact: \_\_\_\_\_
- Visiting Scientist: NWRC Representative/Contact: \_\_\_\_\_

**SIGNATURE**

**DATE**

Concur: <b>NWRC Research Project Leader:</b>		
QAU Review and Processing: <b>NWRC Quality Assurance:</b>		
Concur: <b>NWRC Assistant Director:</b>		
Approved: <b>NWRC Director:</b>		

## REGULATORY CONSIDERATIONS

### **Analytical Chemistry**

Will chemical analysis be required of the NWRC Chemistry Lab Unit?

No  Yes – **Attach the Analytical Chemistry Appendix.**

Will the services of the NWRC Formulation Scientist be needed?

No  Yes – **Attach the Formulation Support Appendix.**

### **Animal Use**

Will the study include the use of animals?

No  Yes – check all that apply below.

- Live animals will be used at an NWRC facility. **Attach the Animal Use Appendix.**
- Handling animals or manipulating the behavior of wildlife in the field. **Attach the Animal Use Appendix.**
- Collaborating institution is responsible for all or part of live animal phase. **Attach the collaborating institution's protocol and IACUC approval.**
- Study will be conducted using privately owned animals. **Attach "Consent for the Use of Privately Owned Animals" form (SOP AD025).**
- No manipulation of the behavior of wildlife in the field (observation only). **No appendix needed.**
- Samples or data opportunistically collected from ongoing operational activities. **No appendix needed.**

### **Biological Laboratories (BioLabs) Support**

Do you anticipate you will require space, equipment, or personnel from the NWRC Biological Laboratories Unit?

No  Yes – **Date of consult with Laboratory Specialist:** [Click here to enter text](#)

### **Microbiological/Biohazardous Materials**

Will any Microbiological/Biohazardous Materials be used?

No  Yes – **Attach the Microbiological/Biohazardous Materials Use Appendix.**

### **Intellectual Property (IP) Considerations**

Do any of these situations apply to this study?

- The condition of confidentiality between you and your collaborator would facilitate open discussions and collaboration.
- This research involves the exchange or transfer of material(s) between the NWRC and your collaborators.
- This research includes existing IP and/or could lead to the development of new IP.

No  Yes – Consult the NWRC Technology Transfer Coordinator. **Date of consult:** May 1, 2020

### **Federal Environmental Statute Considerations**

Will this activity involve a field component and meets any of the following conditions?

The field component will occur on Federal land, is funded with Federal money, and/or involves Federal personnel.

No  Yes

- Complete and **Attach the Endangered Species Act Appendix (ESA)** and
- Complete and attach the **National Environmental Policy Act Appendix (NEPA).**

### **Regulated Product Registration Considerations**

Does this activity involve the transfer OR testing of any pesticide, vaccine, drug, diagnostic kit, or pest control or medical device, or their components, including products still in the research and development stage?

No  Yes - Consult with the NWRC Registration Manager regarding any regulatory requirements.

As determined during this consultation, check the applicable regulatory standards.

none  EPA GLP  FDA CVM GLP  USDA CVB GLP-like  OECD GLP

other: [Click here to enter text](#)



## DESCRIPTION OF ACTIVITIES

### NWRC Study Personnel:

Name	NWRC Project	Contribution to study
Robert T. Sugihara	Island Invasives, Hawaii	Study director, study oversight, lab trials, data summary, reporting
Steve Hess	Island Invasives, Hawaii	Administration, protocol review, supervision and oversight, report review
Israel Leinbach	Island Invasives, Hawaii	Protocol review, lab trials, data input and summary, animal care
Tom McAuliffe	Island Invasives, Hawaii	Lab trials, animal care
Shane Siers	Island Invasives- Guam	Protocol review, report review
Emily Ruell	Registration Unit	EPA study outline, study protocol and report review
Are Berentsen	Rabies Project	Study protocol and report review

### Non-NWRC Affiliates:

Name	Affiliation	Contribution to study
Linton Staples	Animal Control Technologies Australia (ACTA)	Supply fish-based diphacinone bait formulation
Craig Riekena, Chris Thomas	Bell Labs	Supply technical diphacinone

### Sponsor Representative:

Name	Address	Activities at this location
Jeanette O'Hare, Registration Manager	4101 LaPorte Ave., Fort Collins, CO 80521	Review of protocol, any amendments, and final report

### Testing Facilities:

Name	Address	Activities at this location
NWRC Hawaii Field Station	210 Amau'ulu Rd., Hilo, HI	Conduct all phases of laboratory bait efficacy trials with mongooses

### Study Schedule:

Proposed experimental start date: July 6, 2020  
 Proposed termination date: October 30, 2020  
 Proposed study completion/archive date: December 31, 2020

### Background/Justification:

Small Indian mongooses (*Herpestes auro-punctatus*), introduced to Hawaii, Puerto Rico, the U.S. Virgin Islands, and numerous other sites worldwide, are serious predators of native wetland, seabird and upland forest avian species (Nellis and Everard 1983; Yamada and Sugimura 2004; Hays and Conant 2007). Mongooses are well established across most of

the main Hawaiian Islands (Hawaii, Oahu, Maui and Molokai) where they pose a threat to the eggs and nestlings of native ground-nesting birds (Hays and Conant 2007). The threat of accidental or intentional introductions to other mongoose-free islands in the Hawaiian chain (e.g. Kauai, Lanai) and other Pacific locations highlights the need for a comprehensive array of control techniques, including attractive and palatable baits and effective toxicants, to quickly respond to reported sightings or incipient mongoose populations (Pitt et al. 2015; Phillips and Lucey 2016; Berentsen et al. 2018). Mongooses also present a health risk to humans as hosts of leptospirosis in Hawaii (Wong et al. 2012) and the Caribbean (Everard 1976), and as a rabies reservoir on several islands in the Caribbean (Seetahal et al. 2018).

Various strategies have been used to reduce or remove mongoose populations in Hawaii and elsewhere, including trapping and toxic baits. Trapping has been useful in reducing mongoose populations and predation in and around targeted sensitive native areas (ground-nesting upland and seabird colonies). Trapping, however, is labor-intensive, expensive, and only removes mongooses from a limited area (Barun et al. 2011, Sugihara et al. 2018, Berentsen et al. 2018). Toxic baits can provide a more effective and longer-lasting approach to eradicate mongooses from a larger area.

Earlier studies by Keith et al. (1989) found diphacinone to be highly toxic to mongooses with a lethal dose (LD<sub>50</sub>) of 0.18mg/kg body weight. Successful lab and field efficacy trials with diphacinone formulated in a fresh meat bait culminated in a local registration (SLN Reg. No. HI-91004, EPA Reg. No. 12455-9). The SLN label allowed registered applicators to formulate 0.00025% (2.5ppm) of diphacinone in fresh ground beef placed in tamper-proof bait stations deployed in the field to protect ground-nesting native birds. At the registered concentration (0.00025%) the fresh bait had to be maintained in bait stations over an extended period (up to 14 days) to cause mortality by multiple days of feedings by mongooses. The logistics of applicators having to prepare fresh bait formulations regularly, limited bait longevity and other constraints resulted in discontinuance of the SLN registration, mainly due to limited use (Sugihara et al. 2018).

Two commercial diphacinone rodenticide bait products were subsequently approved for mongooses. The rodenticide baits, co-labeled for rats and mongooses, were formulated at 0.005% (50ppm) active diphacinone, the active concentration of most diphacinone baits registered for rats and mice. “Eaton’s® All Weather Bait Blocks Rodenticide with Fish Flavorizer™” (0.005% diphacinone, SLN Reg. No. HI-97-007, EPA Reg. No. 56-44) and “Ramik® Mini Bars Kills Rats and Mice” (0.005% diphacinone, SLN Reg. No. HI-98005, EPA Reg. No. 61282-26) are both hard, waxy, grain-based, bait blocks used in bait stations to control rats and mice. The Eaton’s bait was eventually discontinued in 2004 due to rapid deterioration in the hot and humid environment in Hawaii and concerns of viable exotic plant seeds in the bait matrix (R. Sugihara, *pers. comm.*). The efficacy of the Eaton’s bait was variable in limited field data, suggest that this bait was less successful in areas with low mongoose density or high alternative prey density (Smith et al. 2010).

Recent WS-NWRC cage feeding trials (QA-2196) of several commercial rodenticide baits indicated that the inefficacy of commercial rodenticide formulations to mongooses was likely due to the hard consistency of grain-based pellets and blocks which are not appropriate to the dentition and feeding modes of mongooses. The registered Ramik diphacinone bait block had a fairly low efficacy (20% mortality) over a 5-day feeding period in a laboratory no-choice efficacy trial, which was likely due to low palatability and consumption of the bait rather than low toxicity to mongooses (Sugihara et al. 2018). The Ramik product remains the only registered toxicant bait available for mongoose control in the US, and this registration is state limited to Hawaii.

As part of the QA-2196 trials, technical diphacinone along with other candidate toxicants was formulated in fresh raw chicken, a more attractive bait matrix than the hard rodenticide bait blocks and offered to mongooses in similar 5-day feeding trials. At a concentration of 0.005% (50ppm), the normal dosage of commercial diphacinone-based rodenticide baits, technical diphacinone formulated in raw minced-chicken was found to be highly palatable to mongooses with 100% daily consumption of the fresh bait offered. The overall mortality rate was 70% for mongooses after a single day of feeding and 100% for mongooses over a 3-day feeding period. In cooperation with Japanese researchers attempting to control mongooses on Okinawa and Amami-Oshima, Japan, the 50ppm diphacinone minced chicken bait was found to be equally efficacious for mongooses in lab cage and field enclosure trials conducted in Okinawa (R Sugihara, 2016 and 2018 Japan trip reports). Subsequent experimental field trials with the diphacinone-minced chicken bait was conducted on Amami-Oshima in isolated locations along steep terrain where trapping was not feasible. Preliminary results show that the diphacinone bait was successful in eliminating the remnant mongoose population from the baited areas (T Jogahara, University of Okinawa, *pers. comm.*). This demonstrates the potential for optimizing the susceptibility of diphacinone to mongoose in another more palatable bait matrix with a reduced bait exposure period (Sugihara et al. 2018).

Development of an effective mongoose diphacinone bait will require a softer, palatable, more durable bait matrix that is longer lasting in the field than fresh raw meat. A recently completed lab study (QA-2832) evaluated the palatability of four

candidate non-toxic bait matrices for mongooses to determine which had adequate palatability (are consumed in sufficient amounts) to warrant future consideration as a diphacinone bait matrix. The selected candidate bait matrix was the non-toxic version of a commercial predator bait in Australia called FOXSHIELD<sup>®</sup>, which is a preserved, semi-soft, fish-based cylinder bait encased in a sausage-type skin. FOXSHIELD is produced by Animal Control Technologies (Australia) Pty Ltd (ACTA) Pty Ltd in Somerton, Victoria, Australia (EPA Establishment No.: 091731-AUS-001) for invasive fox control. The non-toxic FOXSHIELD bait matrix was easy to handle and readily consumed by mongooses in the cage feeding trials (QA-2879).

Additionally, a toxicant registration evaluation was recently conducted for mongooses in Hawaii by WS-NWRC (Ruell et al. 2018). Of the four toxicants evaluated, a diphacinone bait for mongooses would likely be the least expensive and fastest candidate to be reviewed and approved for mongoose control by the regulatory agencies, largely due to the abundance of registered diphacinone products and the supporting registration data already available for diphacinone.

The Environmental Protection Agency (EPA) requires laboratory efficacy data for vertebrate pesticide products in accordance with EPA OPPTS 810.1000 guidelines to support the issuance of a future Experimental Use Permit (EUP) for a larger field efficacy study and a subsequent full registration application. Building on the promising results from these previous studies, this proposed two-choice laboratory efficacy study of a bait consisting of the fish-based FOXSHIELD bait matrix containing 0.005% diphacinone continues the momentum toward the eventual goal of field deployment of an effective toxic bait for mongoose control in agriculture, biosecurity, and conservation applications.

#### **Research Objective/Hypothesis:**

The objective is to evaluate the two-choice laboratory efficacy of a fish-based 0.005% (50ppm) diphacinone test bait for mongooses.

#### **Methods, Procedures and Experimental Design:**

**Note:** Two separate rounds of trapping and testing (20 mongooses in each round, 10M:10F, 5M:5F per test group) will be conducted due to caging/housing space and labor constraints.

##### **1) Pre-test and two-choice test diets**

**Pre-test diet (maintenance diet):** Commercial dry cat food - Brand X (to be determined)

**Challenge diet:** Commercial dry cat food - Meow Mix<sup>®</sup> (The J.M. Smucker Co., Decatur, Alabama)

**Toxic bait:** Fish-based bait for mongooses (active ingredient: 0.005% (50ppm) diphacinone, CAS # 82-66-6)

**Manufacture, handling, and characterization of test diets:** The toxic fish-based bait for mongooses (0.005% diphacinone) will be manufactured at Animal Control Technologies (Australia) Pty Ltd in Somerton, Victoria, Australia (EPA Establishment No.: 091731-AUS-001). The batch manufacturing sheet for the toxic bait will be included in the final report. A temperature and relative humidity data logger will accompany the bait during shipment to the NWRC Hawaii Field Station in Hilo, Hawaii.

The pre-test diet (Brand X dry cat food) and the challenge diet (Meow Mix dry cat food) will be purchased from a commercial pet food supplier.

The pre-test diet, challenge diet, and toxic bait will be stored separately at the NWRC Hawaii Field Station. A temperature and relative humidity data logger will accompany each diet in storage. Preparation, weighing and storage of the pre-test and challenge diets will be conducted in a separate "clean" room away from the toxic bait or other chemicals. Stringent controls will be in place to prevent cross-contamination between the toxic bait and challenge diet during handling and cleaning of feeders. In addition, 5-10 grams of the pre-test diet, toxic bait, and challenge diet will be sampled weekly and stored in a -20°C freezer.

The % w/w diphacinone in the toxic bait will be characterized by the NWRC Chemistry Unit in Fort Collins, Colorado in accordance with FIFRA GLP Standards under a separate protocol. A GLP Certificate of Analysis for the toxic bait will be included in the final report.

The pre-test and challenge diet samples will only be tested for potential contamination with diphacinone if any of the control animals show symptoms of anticoagulant poisoning or die during the test period.

## 2) Test animals

Species and type	Small Indian mongoose ( <i>Herpestes auropunctatus</i> ), wild caught
Numbers and sex	Treated group: 20 (10M:10F) Control group: 20 (10M:10F)
Body weight range 1 week prior to trial	Males: 600-800 grams Females: 350-500 grams
Age	Adult
Source	Wild caught in forested habitat, Hilo, Hawaii

**Animal acquisition:** Forty-eight (24 males:24 females) will be captured from forested habitat in Hilo, Hawaii, island of Hawaii following SOP AC 005.01 (revised)- “Capturing, handling, housing and care of mongooses at the Hawaii Field Station”. A total of forty (20 males:20 females) mongooses (*Herpestes auropunctatus*) with body weights ranging between 600-800 grams for males and 350-500 grams for females will be used in the trial across the two rounds of testing (see “Note” under the header “Methods, Procedures and Experimental Design”). The other eight individuals are spares in case any animals are deemed unusable during the pre-test period.

## 3) Pre-test period procedures

**Individual identification:** Upon arrival at the NWRC Hawaii Field Station, each animal will be assigned an individual identification (ID) number.

**Animal housing:** Animals will be individually housed in a 42 cm (tall) x 61 cm (wide) x 64 cm (deep) grated-bottom, stainless steel, modified rabbit-type cages (3904 cm<sup>2</sup> floor area) in the same laboratory room used for the two-choice efficacy trial. Each cage will be assigned a unique number that corresponds with the animal’s ID number.

**Environmental conditions:** Laboratory environmental conditions will be within the range of 20-25 °C, with a light cycle of 12 hours light:12 hours dark (lights on from 0600 to 1800 hours). The NWRC Hawaii Field Station laboratories typically range from 75 to 90% humidity; like what mongooses are naturally exposed to in the wild.

**Acclimation period:** Animals will be acclimated to the laboratory conditions for at least 7 consecutive days (no more than 28 consecutive days) during the pre-test period (Table 1).

During the last 3 days of the acclimation period, animals will be provided food in two identical SS shield feeders on opposite sides of the front of their cages, which will be used to feed the animals for the rest of the trial (Table 1). Instead of metal or ceramic dish feeders, disposable plastic feeder cups within metal feeder shields (Figure 1; Unifab®, Portage, MI), or similar types will be used. These feeder systems have custom shield sizes to prevent animals from nesting in feeder dishes. They are also designed to reduce spillage and cross contamination of the two diets offered to the treated group, which makes weighing uneaten food much easier.

**Pre-test period diet:** Animals will have ad libitum access to the pre-test diet (approximately 70 grams per day), supplemented by 50 grams of previously frozen boneless raw chicken parts once every 4 days (Table 1). Based on past study experiences maintaining wild-caught mongooses in captivity, some caged mongooses will not feed sufficiently on the commercial dry cat food diet alone over multiple days and require supplementation with meat products to maintain body weight and health. The pre-test diet will be split evenly between the two SS shield feeders once those are installed in the cage during the last 3 days of the acclimation period.

**Drinking water:** Animals will have ad libitum access to drinking water (tap water treated and tested for human consumption).

**Health and mortality checks:** Each animal will be checked for visible symptoms or mortality in person at least once each afternoon/evening.

**Animal weighing:** Animals will all be weighed on the same day within 3 days prior to the beginning of the test period.

#### 4) Two-choice test period procedures

**Assignment to treatment groups:** Animals will be randomly assigned to the two treatment groups, stratified by sex, while ensuring that there is no size bias among groups. Method of randomization will be recorded and reported.

**Two-choice test period duration:** The two-choice test period will last 3 consecutive days even if all animals in the treated group succumb to the toxic bait before the end of the two-choice test period (Table 1).

**Test diet feeder locations:** Each morning of two-choice test period, each animal in the treated group will be offered two feeder dishes, one containing the toxic bait and the other containing the challenge diet (Table 1). The two diets will be offered in separate identical SS shield feeders on opposite sides of the front of the cage. Each day of the two-choice test period, the positions of the two feeders in each cage will be reversed from their positions the previous day to offset possible feeding position preferences of mongooses. The control group will be provided with challenge diet in both feeders each day. The location of the two feeders at the front of each cage will also be switched each day.

**Test diet amounts:** At least 70 grams of toxic bait and 70 grams of challenge diet will be available to each individual in the treated group per day during the two-choice phase. At least 70 grams of the challenge diet will be offered to each animal in the control group in each of two identical feeders (70 grams per feeder). No supplemental boneless raw chicken will be offered during the two-choice test period.

**Drinking water:** Water will be provided ad libitum throughout the test period.

**Daily consumption measurements:** The amount of each diet consumed by each animal will be measured approximately every 24 hours during the two-choice test period (Appendix 1). The recorded amount consumed will not include any spilled food, which will be collected and dried (if necessary) before weighing.

After daily food weighing, the test diet in each feeder will be completely replaced with fresh test diet of the same type (70 grams per feeder). The feeder will be cleaned first if it becomes fouled by urine or feces.

**Health and mortality checks:** Animal health and mortalities will be checked twice daily between 8:00am-11:00am and 3:00pm-4:00pm throughout the test period, and symptoms will be recorded in the animal health log (Appendix 2). Dead mongooses will be removed daily or more frequently as observed, weighed, placed in individual labeled (date, weight, sex) plastic bags and stored in the freezer (-29 °C). There will be no euthanasia performed during the 20-day combined two-choice test and post-test periods.

**Trial termination criteria:** If greater than 10% mortality occurs in the control group during the 20-day combined two-choice test and post-test periods, the trial will be discontinued, and the results negated.

#### 5) Post-test period procedures

**Post-test period duration:** The post-test period will be maintained for 17 consecutive days for all surviving animals (Table 1). We will continue to monitor control mongooses for the entire combined 20-day two-choice test and post-test periods, regardless of whether all of the treated group animals die before that time.

**Challenge diet amounts:** Each morning of the post-test period, both feeders in each cage in both the treated and control groups will be filled with 70 grams of challenge diet (Table 1). We will resume offering a supplement of 50 grams of boneless raw chicken every 4 days during this phase.

**Drinking water:** Drinking water will be provided ad libitum throughout the post-test period.

**Daily consumption measurements:** The amount of challenge diet and supplemental chicken consumed by each animal will be measured approximately every 24 hours during the post-test period (Appendix 1). The recorded amount consumed will not include any spilled food, which will be collected and dried (if necessary) before weighing.

After daily food weighing the challenge diet in each feeder will be completely replaced with fresh challenge diet (70 grams per feeder). The feeder will be cleaned first if it becomes fouled by urine or feces.

**Health and mortality checks:** Animal health and mortalities will be checked twice daily between 8:00am-11:00am and 3:00pm-4:00pm throughout the test period, and symptoms will be recorded in the animal health log (Appendix 2). Dead mongooses will be removed daily or more frequently as observed, weighed, placed in individual labeled (date, weight, sex) plastic bags and stored in the freezer (-29 °C). There will be no euthanasia performed during the 20-day combined two-choice test and post-test periods.

**Trial termination criteria:** If greater than 10% mortality occurs in the control group during the 20-day combined two-choice test and post-test periods, the trial will be discontinued, and the results negated.

On the day following the post-test period, all remaining mongooses will be humanely euthanized, weighed and carcasses placed in labeled plastic bags and stored in the freezer (-29 °C).

## 6) Reporting and evaluation of results

**Final report contents:** All individual data and summary statistics (e.g., means and standard deviations) on bodyweights, bodyweight changes, food consumption, symptoms observed during the twice daily health checks, day of the trial that death occurred, and death rate per treatment group will be provided in the final report. Copies of all “raw” data sheets will also be appended to the final report.

**Minimum efficacy criteria:** The efficacy of the toxic bait will be considered acceptable if all the following conditions are met:

- $\geq 33\%$  of the total food consumed by the treated group during the two-choice test period was the toxic bait.
- $\geq 90\%$  of the treated group died during the 20-day combined two-choice test and post-test periods.
- $\leq 10\%$  of the control group died during the 20-day combined two-choice test and post-test periods.

**Table 1. Trial period and accompanying feeders and test diet schedule**

Trial period	Feeders and test diets
Pre-test (acclimation) period (7-28 days)	Both treated and control groups: <ul style="list-style-type: none"> <li>• In-cage feed hopper of pre-test diet, supplemented with boneless raw chicken once every 4 days</li> <li>• <i>During the last 3 days of the pre-test period:</i> 2 identical SS shield feeders of pre-test diet, supplemented with raw chicken once on the day prior to the two-choice test period</li> </ul>
Two-choice test period (3 days)	Treated group: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders containing either toxic bait or challenge diet, alternating the location of the toxic bait and challenge diet feeders each day</li> </ul> Control group: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders, both containing challenge diet, alternating the location of the two feeders each day</li> </ul>
Post-test monitoring period (17 days)	Both treated and control groups: <ul style="list-style-type: none"> <li>• 2 identical SS shield feeders, both containing challenge diet, supplemented with boneless raw chicken once every 4 days</li> </ul>
End of trial	

**Figure 1. SS Shield Feeders**



**Human Health and Safety Risk/Hazard Assessment:**

This protocol poses no unusual risks to health and human safety. The product used in these trials will be handled in accordance with the manufacturer’s instructions and material safety data sheets. Personal protective equipment as prescribed by SOPs HS 004.00- Personal protective equipment and AC 005.01 (revised) will be used when working with live mongooses.

**Standard Operating Procedures (SOPs)/Analytical Chemistry Methods:**

SOP/Method No.	Title
ACHI 002.01	Euthanasia with carbon dioxide (CO <sub>2</sub> ) gas at the Hawaii Field Station
AC 005.01, revised	Capturing, handling, housing and care of mongooses at the Hawaii Field Station
HS 004.00	Personal protective equipment
ACCO 002.00	Animal handling to maintain secure identification

**Cost Estimate for Each Fiscal Year:**

	FY-20	FY-21
A. Salary and Benefits	\$30,000	\$10,000
B. Facilities (in addition to existing facility or space costs)		
C. Equipment		
D. Supplies	\$2,500	
E. Animal Care Costs		
F. Operating Costs (travel, misc. services, etc.)		
<b>TOTAL</b>	<b>\$32,500</b>	<b>\$10,000</b>

**List of Records to be Maintained:**

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a. Animal health observation logs
  - b. Animal testing room temperature logs
  - c. Test substance/challenge diet/maintenance diet receipt and formulation records
  - d. Individual daily consumption records
  - e. Animal health checks/disposition logs
  - f. Necropsy log
  - g. Personnel training records
  - h. Animal trapping records
  - i. Reports of analyses
  - j. EPA study outline review
- D. Final Report

**Archiving:**

The protocol, amendments, raw data, documentation, records, 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.

**Protocol Amendments:**

Any changes in this protocol will be documented prior to the change using the Protocol Amendment form, reviewed by the appropriate personnel, signed, and dated. Approved amendments will be distributed to all study participants as appropriate.

**Regulatory Guidelines:**

EPA OPPTS 810.1000: Overview, definitions, and general considerations  
 EPA Pesticide Assessment Guidelines- Subdivision G: Product Performance  
 FIFRA Good Laboratory Practice Standards (GLPs; 40 CFR 160)



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**Other Pertinent Attachments:** (list in order of appearance)

- Appendix 1. Two-choice bait assay data sheet
- Appendix 2. Animal Health Log
- Appendix 3. Animal Use Appendix, Column E Explanation

DRAFT





### ANIMAL USE APPENDIX (Appendix 3)

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. Related Protocols:

*List by number*

QA-2196: Laboratory evaluation of the palatability and toxicity of candidate baits/toxicants for mongooses (*Herpestes auropunctatus*)

QA-2832: Development and testing of a matrix for mongoose toxicant baits: Placebo cage palatability trials

#### B. Assurance of Non-duplication of studies

*Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.*

In February-March 2020 a literature search of BIOSIS and Google Scholar was conducted using combinations of keywords including: *Mongoose, Herpestes, toxicants, palatable, bait matrix, Hawaii*. Besides the single non-published report by Keith et. al. 1989 that summarized the laboratory and field trials of fresh ground beef-diphacinone baiting trials in Hawaii, there were no studies that duplicate our proposed study.

#### C. Staff Qualifications

*All study participants will 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.*

*List the study participants that will be working independently with animals and provide their qualifications/certifications (i.e. name, title, and a brief description of training/experience).*

Steven Hess, Ph.D., Hawaii Field Station Project Leader, Supervisory Research Wildlife Biologist, has 20+ years' experience in conducting field trials on monitoring and evaluating control methods for invasive ungulates and small mammals in Hawaii.

Robert Sugihara, lead biological science technician, has over 35 years' experience in animal handling, wildlife biology and research, to include implementation and supervision of several protocols nearly identical to this one. He will serve as study director. He has directed or assisted with numerous laboratory bait and bait matrix feeding bioassays with rats, mice and mongooses. He has extensive experience in animal care, recording detailed observations, documentation of test procedures, including GLP regulated studies.

Israel Leinbach, Biologist, has an MSc in Biology and 5+ years' experience in field and laboratory science. He has led and assisted in field and laboratory trials with rats, mice and mongooses, including lab cage trials evaluating various baits, bait matrices and field bait longevity studies. He has assisted in conducting, recording observations in animal care of small rodents and mongooses.

Thomas McAuliffe has been employed as a Biological Science Technician with Wildlife Services for ten years and has participated in many operational and research activities in Hawaii, Guam, and other more remote islands in the Pacific Basin. He has assisted in various laboratory feeding bioassays with rats, mice and mongooses, including animal care, detailed record keeping and documentation of test procedures.

#### **D. Training Assurance**

*Provide an assurance that participants have read the protocol (especially those who will handle animals), and have completed appropriate training (e.g., CITI or other training – with documentation).*

All study participants will have read the study protocol and associated SOPs. Additional copies of CITI Lab Animal training documentation for key personnel are 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. Study participants have received formal GLP training in record-keeping and conducting animal trials

#### **E. Permits**

*Provide information related to any permits current in possession or being applied for, which are required for the use of animals related to this research activity.*

A permit (HI-ISL-2017-01) for harboring mongooses for research purposes from the Hawaii Department of Agriculture is current. No other permits are required for this study. Right of access permits/agreements will be obtained as necessary to access sites for capturing mongooses.

#### **Animal Description**

1. Animals:  
Small Indian mongoose
2. Species, subspecies (if applicable):  
Herpestes auropunctatus, wild-captured
3. Number and Sex (known or estimated):  
40 (20 males:20 females), conducted in 2 separate rounds of testing (each round includes 20 animals- 10 males:10 females; 5 males:5 females per test group)
4. Additional contingency animals (number and sex):  
An extra 4 (2 males:2 females) mongooses per round (total=8) are extra contingency animals; total of 48 animals.
5. Acceptable Body weight criteria:  
Males: 600-800 grams (housed and 1 week prior to test period)  
Females: 350-500 grams (housed and 1 week prior to test period)
6. Acceptable Age criteria:  
Adults (Determined by body weight criteria)

#### **F. 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:  
The study is required to demonstrate the efficacy of the fish-based diphacinone test bait for lethal control of the target species and relies on the feeding behavior of the subjects; no non-living models could substitute for living animals.
2. Rationale for appropriateness of the species to be used:  
Mongooses (*Herpestes auropunctatus*) are the target invasive species. EPA requires laboratory efficacy data for the target vertebrate species before approval is granted for further testing (field trial under an Experimental Use

Permit) towards the goal of obtaining a Section 3 registration to control mongooses in Hawaii and elsewhere in the US.

3. Rationale for numbers of animals to be used, including numbers of animal to be obtained as extra if appropriate (e.g. how many additional animals do you intend to hold in reserve to substitute in for animals found to be unfit for experimentation). Also explain how the numbers of animals requested/planned for relates to the analysis on how numbers were determined or how the numbers requested should satisfy the study requirements.

Twenty (20) animals per test group (treated and control groups), n=40 total, is the minimum number of animals allowed by the EPA OPP guidelines to demonstrate efficacy. An additional 8 animals (4 males:4 females) will be obtained to ensure the proper sex ratio and to replace animals that are inappropriate for trials due to health or behavioral issues during the pre-test acclimation period.

#### **G. Source**

*Describe where the animals will be trapped or obtained or identify the vendor by name and address.*

Wild mongooses will be live captured from forested habitats in Hilo, Hawaii, island of Hawaii.

#### **H. Method of identification of animals**

*Explain briefly how animals will be marked or identified to prevent misidentification, and cite any appropriate SOP(s)*

Each mongoose will be assigned and maintained in an individual cage that will have a unique number attached to the cage; that number will correspond to the animal's assigned ID number. Refer to SOP ACCO 002.00 Animal handling to maintain secure identification. No individual animal marking or tagging will be done.

#### **I. Trapping/Collecting**

*Explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.), and cite any appropriate SOP(s).*

Standard wire-cage Tomahawk® live traps (Model 602SS-F) will be set on the ground in protected sites as under vegetation or other cover (shade) to reduce direct exposure to rain and hot sunlight. Traps baited with coconut chunks will be set/activated early in the morning and checked daily before 9:00 am and reset, as necessary. Mongooses forage throughout daylight hours; to minimize stress to trapped mongooses, traps will be check 2-3 times during the day as feasible. At locations with minimal vegetation cover or high potential for non-target captures (rats and mice) during night-time hours, traps will be shut down in late afternoon and reset the next morning.

Any mongoose unsuitable for the intended study (<desired body weight, poor body condition, aggressive behavior, gender skewed, etc.) and non-target captures will be released onsite immediately. Under some circumstances, release of animals may not be allowed at certain sites; in such cases, animals will be transported to the field station for euthanasia (SOP AC/HI 002.01 Euthanasia with carbon dioxide (CO<sub>2</sub>) gas at the Hawaii Field Station). Traps will be rebaited with fresh coconut bait as needed. Refer to SOP AC 005.01 (revised): Capturing, handling, housing and care of mongooses at the Hawaii Field Station.

#### **J. Transport**

*Explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, total transit time, etc., and cite any appropriate SOP(s).*

Mongoosees will be transported individually in their original capture traps. When transporting mongoosees in an open vehicle (pickup truck) during hot (>85° F) or during rainy weather, a tarpaulin or other opaque covering will be placed over the traps to provide shelter and reduce stress, allowing for adequate air ventilation between the cover material and the traps. Alternatively, large-leafed vegetation will be placed over the traps.

Live mongoosees used for trials conducted at the field station are exclusively collected from locations on the island of Hawaii within 1 hour driving distance to the field station. Food and water source will not be provided due to the short transport time from capture location to the research facility. Refer to SOP AC 005.01 (revised): Capturing, handling, housing and care of mongoosees at the Hawaii Field Station.

#### **K. Handling/restraint**

*Explain briefly how the animals will be held or restrained (manual vs. chemical) throughout the study and cite any appropriate SOP(s).*

Most animal processing procedures (weighing, gender identification, transferring mongoosees to holding cages) can be accomplished with the mongoose in its capture trap without direct handling of mongoosees. Heavy leather or chain mesh gloves will be worn to physically restrain the mongoose if needed. Refer to SOP AC 005.01 (revised): Capturing, handling, housing and care of mongoosees at the Hawaii Field Station.

#### **L. Quarantine**

*Explain briefly the procedure for the quarantine of animals and cite the appropriate SOP(s).*

All animals will be maintained in quarantine at the Hawaii Field Station for observation and stabilization for at least 7 days prior to testing, but less than 28 days. Access to the quarantine room shall be restricted to only persons (e.g. study director, principle investigator, animal care technicians, QA and IACUC staff, etc.) whose job descriptions require access in the performance of necessary activities. All animal care and room maintenance records will be stored in a designated area in the animal room at the close of each day. An Animal Identification Card will always be securely attached to each cage. Refer to SOP ACCO 002.00- Animal handling to maintain secure identification.

#### **M. Housing/Caging**

*Explain briefly how housing/caging will be done (including information on feeder animals if used). Provide information regarding special caging or housing requirements, and cite any appropriate SOP(s)*

Animals will be individually housed in 42 cm (tall) x 61 cm (wide) x 64 cm (deep) grated-bottom, stainless steel, modified rabbit-type cages (3904 cm<sup>2</sup> floor area) in the designated animal room(s) at the Hawaii Field Station. Refer to SOP AC 005.01 (revised): Capturing, handling, housing and care of mongoosees at the Hawaii Field Station.

#### **N. Diet/Water**

*Explain briefly how the animals will be fed and watered and cite any appropriate SOP(s). Provide information regarding maintenance diets, special diets, and dietary manipulations, and describe components of any test substance formulations.*

Each day, 70 grams of the test bait and 70 grams of the challenge diet will be offered separately to the treated group mongoosees (two-choice trial) in two identical feeders placed on opposite sides of the front of the cage. Each day, control group mongoosees will also be offered two feeders (identical to those used for the test bait), each containing the challenge diet (70g per container). No supplemental raw chicken will be offered during the two-choice test period. The position (left-right) of the 2 feeder containers placed in the front of the cage will be switched daily during the 3-day bait exposure period to reduce positional feeding bias of individual animals

#### Post-test period

The test bait feeders will be removed at the end of the 3-day test period for the treated group and replaced with new or thoroughly washed feeders holding 70 grams of the challenge diet (2 feeders of 70 grams each). All remaining



mongooses in both the treated and control groups will continue to be fed the challenge diet (2 feeders of 70 grams each) for an additional 17 consecutive days, with the challenge diet consumption weighed, recorded, and replaced daily as described above. Mongooses will be supplemented with raw chicken (placed on the cage floor) every 4 days.

**O. Monitoring**

*Describe how animals will be monitored while on test, especially those who are involved in a toxicity or disease study, or have been injected with a test substance, etc.*

Animals will be examined at least two times daily between 8:00am-11:00am and 3:00pm-4:00pm by the study director or designee, with notes of animal condition recorded in the Animal Health Log (Appendix 2).

**P. Study End Point:**

*Describe how the end of the activities which involve the use of animals is determined.*

The study end point for each animal will be survival of the treatment and post-treatment monitoring period or death by diphacinone poisoning or other causes. No euthanasia will be conducted during the test period. Surviving mongooses will be euthanized at the end of the 20-day trial period (at the end of the 17-day post-trial monitoring period).

**Q. Disposition of animals**

*Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study and cite any appropriate SOP(s).*

Mongooses that did not expire during the 3-day two-choice period or the 17-day post-trial monitoring period will be euthanized using carbon dioxide overdose (SOP ACHI 002.00- Euthanasia with carbon dioxide (CO<sub>2</sub>) gas at the Hawaii Field Station) at the completion of the trials. All carcasses will be frozen and later disposed-of at the County of Hawaii sanitary landfill.

**R. 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: Laurie Baeten (NWRC), Alfred Mina (local)

Date of Consultation: May 12, 2020

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

- No
- Yes - Continue with the following items.

a) Alternative procedures:

*Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.*

No alternative procedure could provide the same level of assurance of efficacy as the proposed protocol.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

*Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.*

No sedatives, analgesics or anesthetics will be administered, and no animal will be euthanized to relieve pain or distress during the test period. See Column E explanation.

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

c) Surgery:

*Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.*

N/A

#### **S. Euthanasia**

*Describe the appropriate method of euthanasia to be used (cite the current AVMA Guidelines, appropriate SOP, or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)*

Sick or injured animals may be euthanized during the pre-test period and replaced with the spare animals that will be obtained for this study. Sick animals will not be euthanized during the two-choice test or post-test period without negating the results of the study. Euthanasia can be considered for documented severe injuries that occur during the two-choice or post-test period. See Column E explanation (Section 5).

At the end of the study, all animals will be euthanized by carbon dioxide as described in SOP ACHI 002.00- Euthanasia with carbon dioxide (CO<sub>2</sub>) gas at the Hawaii Field Station and as stated in the AVMA guidelines.

#### **T. IACUC Approval**

Date of IACUC Approval Letter: \_\_\_\_\_

## COLUMN E EXPLANATION

**Note: This is used as additional justification required for studies which involve unrelieved pain and distress in animals. It is an annual APHIS reporting requirement for regulated facilities.**

1. **Registration Number:** N/A

2. **Number of animals used in this study during this reporting period:**

Forty (40) mongooses will be used in this study. Of these, only 20 mongooses (10 males:10 females) will be treated and at risk of unrelieved pain and distress. An additional 8 mongooses will be held as extras for replacing an animals deemed unsuitable during the pre-test period only.

3. **Species (common name) of animals used in study during this reporting period:**

*Herpestes auropunctatus* (Small Indian mongoose), wild-caught

4. **Explain procedure producing pain and/or distress:**

The test substance (Fish-based bait for mongooses, which contains 0.005% (50ppm) diphacinone) will be offered to caged mongooses in free-feeding two-choice trials over a 3-day bait exposure period, along with a non-toxic challenge diet. Intoxication by anticoagulant rodenticides may cause more than momentary pain or distress in some animals.

5. **Provide scientific justification why pain or distress could not be relieved:**

*State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):*

The Environmental Protection Agency (EPA) requires two-choice laboratory efficacy data (product performance data) for vertebrate pesticide products in accordance with EPA OPPTS 810.1000 guidelines to support the issuance of a future Experimental Use Permit (EUP) for a larger field efficacy study and a subsequent full registration application. The study must be conducted under controlled conditions: individually caged animals in a climate-controlled animal room. The study also requires relatively normal metabolic and physiological processes to be occurring in the animals. Hence, sedatives or analgesics are not appropriate because they may affect--and in particular, slow down--those metabolic processes.

It is not known to what extent anticoagulants cause pain or distress in treated animals and the scientific literature does not provide consistent answers on this matter. Mongooses feeding on anticoagulant baits must generally do so for multiple days because of the low concentrations and the slow action of anticoagulants in the body, which ultimately cause increased internal hemorrhaging over a few days' time after a large enough dose is consumed. The animals continue to be active and feed for several days after consuming a lethal dose. Eventually, they become more lethargic and stop feeding. Death usually results soon thereafter, however, some animals have been documented to recover despite exhibiting symptoms of anticoagulant poisoning (Appendix 3). Signs and symptoms can range from no observable symptoms to severe.

Animals will be examined at least two times daily (morning and evening) by the study director or designee, with notes of animal condition recorded in the animal health log (Appendix 2). Sick or injured animals may be euthanized during the pre-test period and replaced with the spare animals that will be obtained for this study. Sick animals will not be euthanized during the two-choice test or post-test period without negating the results of the study. Euthanasia can be considered for documented severe injuries that occur during the two-choice or post-test period.

6. **What, if any, federal regulations require this procedure?**

**Agency:** U.S. Environmental Protection Agency

**CFR:** 40 CFR 158.400 (Subpart E – Product Performance)  
Product Performance OPPTS 810.1000

DRAFT

## ENDANGERED SPECIES ACT (ESA) APPENDIX

All activities or programs that are authorized, funded, or carried out, in whole or in part, by federal agencies in the U.S. or upon the high seas are regulated under the ESA. This includes research activities authorized, funded, or conducted by federal agencies and employees.

Before any field activity can take place you must assess the potential effects the proposed action could have on species, populations, or critical habitat protected under the ESA, and then make “effects determinations”. Finally, you must maintain an administrative record (i.e., documentation of the evaluation) for the field activity under the ESA.

This appendix will help you document your effects determinations for this action, and determine whether further consultation with the U.S. Fish and Wildlife Service (USFWS) and/or National Marine Fisheries Service (NMFS) is required under section 7 of the ESA.

**This appendix does not cover regulatory requirements for state listed species. You must determine those by contacting the State agency responsible for wildlife management.**

### Links to USFWS/NMFS Resources on Effects Determinations

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[Effects Determination Guidance \(NMFS\)](#)

[Effects Determination Step-by-Step Instructions \(USFWS\)](#)

[USFWS Consultation Handbook](#)

### Effects Determinations Instructions and Decision Tool

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1. Is another federal agency taking care of the section 7 responsibilities under ESA for this field activity?
- Yes      Go to #5, check the box, and follow the instructions.
- No        Go to #2.
- 

2. **Read all of the instructions under I, II, and III below in order to answer this question!**

I. Determine the action area, which includes the area where the field activity will actually occur and all areas that reasonably could be directly or indirectly affected by the field activity immediately or in the future.

II. Go to: [USFWS IPaC online planning tool](#) (Hold Ctrl + Click on blue link), click and follow the instructions to map your action area determined in Step I. Then request an “official species list” under “Regulatory Documents” ([instructional video](#); Hold Ctrl + Click on blue link). The official species list will be emailed to you. This official species list will include all species, experimental populations, and critical habitat protected under the ESA that occur in your action area.

*Note: Only consider resources protected under the ESA for this appendix (e.g., do not include species protected under the Migratory Bird Treaty Act or the Bald and Golden Eagle Protection Act).*

III. Based on the results from Step II, do any threatened, endangered, or proposed species (animals and plants), experimental populations, or designated or proposed critical habitat protected under the ESA occur in your action area?

- Yes    Then go to #3.
- No     Go to #6, check the box, and follow the instructions.
-

3. **Read all of the instructions under I, II, and III below in order to properly fill out the table below.**

I. Assess all potential effects of the proposed action **on each** protected species, experimental population, or critical habitat that occurs in your action area by doing the following:

- a. Identify all potential stressors resulting from the action to one or more individuals of the species and/or to “primary constituent elements” of the species’ habitat; and
  - *Primary constituent elements include: 1) space for individual and population growth, and for normal behavior, 2) food, water, air, light, minerals, or other nutritional or physiological requirements, 3) cover or shelter, 4) sites for breeding, reproduction, rearing of offspring, germination, or seed dispersal, and 5) habitats that are protected from disturbance or are representative of the historic geographic and ecological distributions of a species.*
- b. Evaluate all potential pathways in which one or more individuals of the species and/or primary constituent elements of the species’ habitat could be exposed to those stressors, including the potential intensity, frequency, and duration of the exposure.

When doing this, you must consider all of the following types of potential effects:

- Direct effects: Changes that occur during implementation of the action.
- Indirect effects: Changes that occur after implementation of the action (at any point in time).
- Interrelated effects: Changes that are the result of a larger action and depend on the larger action for their justification.
- Interdependent effects: Changes that are the result of other actions that would not occur without the action under consideration.
- Cumulative effects: Changes that are the impact of future activities (federal, state, and private) that are reasonably certain to occur after the action has occurred.

II. Then:

**A) For the following ESA protection status classifications:**

- **Threatened species**
- **Endangered species**
- **Designated critical habitat**
- **Essential experimental population**
- **Non-essential experimental population (inside of a National Park or National Wildlife Refuge)**

a) Determine whether those potential effects are:

- Zero: No potential for exposure to a stressor.
- Beneficial: Effects are immediate and wholly positive.
- Insignificant: Effects relate to the size of the impact and should never reach the scale where “take” occurs. Based on best judgment, a person would not be able to meaningfully measure, detect, or evaluate insignificant effects.
  - *Take includes intentional or incidental harassment, trapping, capture, injury, or death, or otherwise changing the behavior of an individual of a protected species in a way that negatively impacts their fitness, reproduction, or survival, or damaging or altering designated critical habitat.*
- Discountable: Based on best judgment, a person would not expect these effects to occur, because they are extremely unlikely (this must be justified).
- Adverse: All other effects are adverse effects. Take must be considered an adverse effect.

b) Identify potential [mitigation](#) or conservation measures that can be taken to potentially reduce an adverse effect to an insignificant or discountable effect.

*Note: A mitigation measure cannot reduce an insignificant, discountable, or adverse effect to zero effect.*

c) Make the appropriate effect determination for the species, experimental population, or critical habitat:

- **No effect (NE):** The proposed action will have no impact, because there is zero potential for exposure to a stressor resulting from the proposed action (e.g., the species uses completely different habitat units than those directly or indirectly impacted by the action, or is seasonally absent and primary constituent elements of its habitat will not be affected).
  - *Any potential beneficial, insignificant, discountable, or adverse effects of the action means you cannot make an NE determination, even when the potential effects are improbable.*
  - *You also cannot mitigate to an NE determination, but you can move the location of your field activity to another site where the species or critical habitat will have zero exposure to a stressor resulting from the action and then make an NE determination.*
- **May affect, but not likely to adversely affect (NLAA):** Only applies if the potential effects of the proposed action are wholly beneficial, insignificant, or discountable.
  - *Any potential take resulting from the action means you cannot make an NLAA determination, even when the take is improbable.*
- **May affect, and is likely to adversely affect (LAA):** Applies if the proposed action has the potential to cause adverse effects.
  - *You can potentially mitigate to reduce an LAA to an NLAA determination.*

Or:

**B) For the following ESA protection status classifications:**

- **Proposed species**
- **Proposed critical habitat**
- **Non-essential experimental population (outside of a National Park or National Wildlife Refuge)**

a) Determine whether those potential effects will:

- **Not likely to jeopardize/adversely modify:**
  - A) The proposed action is not likely to reduce the reproduction, numbers, or distribution of the proposed species or the non-essential experimental population in a way that would reasonably be expected to directly or indirectly reduce appreciably the likelihood of both the survival and recovery of that species; or
  - B) The proposed action is not likely to adversely modify the proposed critical habitat.
- **Likely to jeopardize/adversely modify:**
  - A) The proposed action could reasonably be expected to directly or indirectly appreciably reduce the likelihood of both the survival and recovery of the proposed species or the non-essential experimental population by reducing reproduction, numbers, or the distribution of that species; or
  - B) The proposed action is likely to adversely modify the proposed critical habitat.

III. Finally, **for each ESA-protected resource** record in the table below: **a)** the name, **b)** the protection status, **c)** the appropriate effect determination, and **d)** an explanation/rationale/justification for the effect determination for each species (including mitigation measures, if applicable), experimental population, or critical habitat in your action area. Archive all supporting documentation (e.g., USFWS informational resources, peer-reviewed publications, survey data). Once you have completed the table, go to #4.

**a. Name of species/experimental population/critical habitat:**  
 Nene (*Branta sandvicensis*) Hawaiian goose (USDA field station and Kilauea Military Reservation sites only)

**Select the species' ESA protection status and your effect determination below** (complete only one column of this section)

- b. ESA protection status:**
- Threatened species
  - Endangered species
  - Designated critical habitat
  - Experimental population (check which one applies below):
    - Essential
    - Non-essential, inside a National Park or Refuge

- c. Effect determination**
- NE (*Note: you cannot mitigate to an NE*)
  - NLAA (*check all that apply below*)
    - All potential effects are either:
      - Beneficial Effects
      - Insignificant Effects
      - Discountable Effects
  - LAA

- b. ESA protection status:**
- Proposed species
  - Proposed critical habitat
  - Experimental population
    - Non-essential, outside of a National Park or Refuge

- c. Effect determination:**
- Not likely to jeopardize/adversely modify
  - Likely to jeopardize/adversely modify

**d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:**  
 In 30+ years of live trapping for mongooses by NWRC in agriculture, urban, mixed-use and conservation areas, zero (0) Nene (*Branta sandvicensis*) have been captured or observed interacting with mongooses or rats captured in traps. The trap type, bait used, trap location and placement/camouflage in heavy canopy or vegetation are unlikely to result in any encounter with these birds. If at any point going forward any changes in these observations occur, we will cease operations and contact USWFS immediately.

**a. Name of species/experimental population/critical habitat:**  
 Hawaiian hawk (*Buteo solitaries*) (All sites)

**Select the species' ESA protection status and your effect determination below** (complete only one column of this section)

- b. ESA protection status:**
- Threatened species
  - Endangered species
  - Designated critical habitat
  - Experimental population (check which one applies below):
    - Essential
    - Non-essential, inside a National Park or Refuge

- c. Effect determination**
- NE (*Note: you cannot mitigate to an NE*)
  - NLAA (*check all that apply below*)
    - All potential effects are either:
      - Beneficial Effects
      - Insignificant Effects
      - Discountable Effects
  - LAA

- b. ESA protection status:**
- Proposed species
  - Proposed critical habitat
  - Experimental population
    - Non-essential, outside of a National Park or Refuge

- c. Effect determination:**
- Not likely to jeopardize/adversely modify
  - Likely to jeopardize/adversely modify



**d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:**

In 30+ years of live trapping for mongooses by NWRC in agriculture, urban, mixed-use and conservation areas, zero (0) Hawaiian hawk (*Buteo solitaries*) have been captured or observed interacting with traps. The trap type, bait used, trap location and placement/camouflage in heavy canopy or vegetation on the ground are unlikely to result in any encounter with these birds perched in the tree top canopy or soaring overhead. If at any point going forward any changes in these observations occur, we will cease operations and contact USWFS immediately.

**a. Name of species/experimental population/critical habitat:**

Hawaiian hoary bat (*Lasiurus cinereus semotus*) (All sites)

**Select the species' ESA protection status and your effect determination below** (complete only one column of this section)

**b. ESA protection status:**

- Threatened species
- Endangered species
- Designated critical habitat
- Experimental population (check which one applies below):
  - Essential
  - Non-essential, inside a National Park or Refuge

**b. ESA protection status:**

- Proposed species
- Proposed critical habitat
- Experimental population
  - Non-essential, outside of a National Park or Refuge

**c. Effect determination**

- NE (Note: you cannot mitigate to an NE)
- NLAA (check all that apply below)
  - All potential effects are either:
    - Beneficial Effects
    - Insignificant Effects
    - Discountable Effects
- LAA

**c. Effect determination:**

- Not likely to jeopardize/adversely modify
- Likely to jeopardize/adversely modify

**d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:**

In 30+ years of live trapping for mongooses by NWRC in agriculture, urban, mixed-use and conservation areas, zero (0) Hawaiian hoary bat (*Lasiurus cinereus semotus*) have been captured or observed interacting with traps. The bat's arboreal behavior makes it unlikely to result in any encounter with traps placed on the ground or personnel checking traps. If at any point going forward any changes in these observations occur, we will cease operations and contact USWFS immediately.

**a. Name of species/experimental population/critical habitat:**

Haiwale (*Cyrtandra nanawaleensis*) native plant, no common name (Kilauea Military Reservation site only)

**Select the species' ESA protection status and your effect determination below** (complete only one column of this section)

**b. ESA protection status:**

- Threatened species
- Endangered species
- Designated critical habitat
- Experimental population (check which one applies below):
  - Essential
  - Non-essential, inside a National Park or Refuge

**c. Effect determination**

- NE (*Note: you cannot mitigate to an NE*)
- NLAA (*check all that apply below*)
  - All potential effects are either:
    - Beneficial Effects
    - Insignificant Effects
    - Discountable Effects
- LAA

**b. ESA protection status:**

- Proposed species
- Proposed critical habitat
- Experimental population
  - Non-essential, outside of a National Park or Refuge

**c. Effect determination:**

- Not likely to jeopardize/adversely modify
- Likely to jeopardize/adversely modify

**d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:**

The native plant species, Haiwale (*Cyrtandra nanawaleensis*), is not found at the targeted trapping location (firing range) within the Kilauea Military Reservation site.. If at any point going forward any changes in these observations occur, we will cease operations and contact USWFS immediately.

**Note:** To add species, experimental populations, or critical habitat: 1) click anywhere in the table cells above, and then 2) click the "+" in the bottom right corner of the cells selected.

**4.** Once you have completed the table above, select the appropriate option below:

- All species, experimental populations, and critical habitat effect determinations are NE or "Not likely to jeopardize/adversely modify". Go to #6, check the box, and follow the instructions.
- One or more species, experimental populations, or critical habitat effect determinations are NLAA, and none of the determinations are LAA or "Likely to jeopardize/adversely modify". Go to #7, check the box, and follow the instructions.
- One or more species or critical habitat effect determinations are LAA or "Likely to jeopardize/adversely modify". Go to #8, check the box, and follow the instructions.

## ESA Appendix Conclusion

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5.  Another federal agency is fulfilling the section 7 responsibilities for this proposed action.

[Click here and cite document](#)

- Do not conduct the requested field activities until no effect determinations have been made by the other agency or consultation/conference with USFWS/NMFS is complete. You must be informed of and follow the requirements of the consultation/conference.
  - **You are finished with the ESA Appendix and your responsibilities under the ESA unless an additional species or critical habitat is protected under the ESA in the action area during the action or if the action area expands.**
- 

6.  A **no effect or not likely to jeopardize/adversely modify** determination is made for all species, experimental populations, and critical habitat protected under the ESA for the proposed action.

- Save and archive your official species list and any other information used to reach this conclusion.
  - **You are finished with the ESA Appendix and your responsibilities under the ESA unless an additional species or critical habitat is protected under the ESA in the action area during the action or if the action area expands.**
- 

7.  The proposed action is **may affect, but is not likely to adversely affect** one or more species, experimental populations, or critical habitat protected under the ESA within the action area.

- The NWRC NEPA contact will initiate the informal consultation process with USFWS/NMFS Ecological Services. **Written concurrence from USFWS/NMFS Ecological Services on the NLAA determination(s) is required before the action may be undertaken, or before an irreversible or irretrievable federal commitment to the action is made.** Correspondence from USFWS Refuge personnel will not suffice. This process usually takes at least 1 month.
  - Save and archive all documents and correspondence, including the official species list and concurrence letter from USFWS/NMFS.
  - **You are finished with the ESA Appendix, but not with your responsibilities under the ESA.**
- 

8.  The proposed action **may affect, and is likely to adversely affect** or one or more species, experimental populations, or critical habitat within the action area, and/or is **likely to jeopardize** the continued existence of proposed species or experimental populations, and/or is **likely to adversely modify** proposed critical habitat.

- Contact the NWRC NEPA contact to initiate a formal consultation and conference process with USFWS/NMFS Ecological Services. **The formal consultation must be concluded before the action may be undertaken, or before an irreversible or irretrievable federal commitment to the action is made.** This process takes a minimum of 6 months.
  - Save and archive all documents and correspondence, including the official species list, the Biological Assessment, Section 10 permits (if applicable), and the Biological Opinion from USFWS/NMFS.
  - **You are finished with the ESA Appendix, but not with your responsibilities under the ESA.**
-

## NATIONAL ENVIRONMENTAL POLICY ACT (NEPA) APPENDIX

This appendix is intended to aid the Study Director with determining whether a proposed project qualifies for a categorical exclusion as allowed by the USDA APHIS Implementing Regulations (7 CFR, part 372). Categorical exclusions are classes of federal actions that do not individually or cumulatively have a significant effect on the human environment.

- **Complete the Endangered Species Act (ESA) Appendix prior to completing this appendix.**
- **This appendix does not cover regulatory requirements for States. You must determine those by contacting the appropriate State agency.**

A. Is another agency completing the NEPA and ESA requirements for the proposed action, and do they adequately address all proposed NWRC activities?

Yes – Please contact the NWRC NEPA Contact to determine the appropriate level of documentation. (A copy of the document must be included when your study is archived).

[Click here and cite document](#)

No – Continue to question B.

B. What was your conclusion in the ESA Appendix?

The proposed action will require a formal consultation with USFWS or the National Marine Fisheries Service (NMFS) – This study does not qualify for a Categorical Exclusion, and **an EA or EIS should be prepared** before initiation of the project. **You are done with this appendix. Contact the NEPA Coordinator for assistance.**

The proposed action will require an informal consultation with USFWS or NMFS – This study may qualify for a Categorical Exclusion if you determined that the proposed action may affect, but is not likely to adversely affect all listed species, experimental populations, or critical habitats **AND** USFWS or NMFS concurs in writing. – Continue to question C.

No consultation (formal or informal) with USFWS or NMFS is required under the ESA – Continue to question C.

C. Do any agency actions classified as undertakings under the National Historical Preservation Act (NHPA) result in adverse effects to historic properties within the area of potential effects (<http://www.achp.gov/flowexplain.html>).

**Undertakings** are projects, activities or programs either funded, permitted, licensed or approved by a Federal Agency. Undertakings may take place either on or off federally controlled property and include new and continuing projects, activities, or programs and any of their elements not previously considered under Section 106 of the NHPA.

**Adverse Effects** occur when an undertaking may directly or indirectly alter characteristics of a historic property that qualify it for inclusion in the Register. Examples of adverse effects include physical destruction or damage; alteration not consistent with the Secretary of the Interior's *Standards*; relocation of a property; change of use or physical features of a property's setting; visual, atmospheric, or audible intrusions; neglect resulting in deterioration; or transfer, lease, or sale of a property out of Federal ownership or control without adequate protections.

Use one of the following links to determine if historical properties fall within the proposed action area:

- a. <https://www.nps.gov/maps/full.html?mapId=7ad17cc9-b808-4ff8-a2f9-a99909164466> (Useful for smaller geographic areas)
- b. <http://nepassisttool.epa.gov/nepassist/entry.aspx> (Useful for larger geographic areas)

Yes – Contact the State Historic Preservation Office (SHPO) for consultation (<http://ncshpo.org/shpodirectory.shtml>). This study may not qualify for a Categorical Exclusion and **an EA or EIS may need to be prepared** before initiation of the project if there are concerns from the SHPO. (A copy of the letter to the SHPO and any other information regarding the consultation must be included when your study is archived). – Continue to question D.

No – Continue to question D.

**D.** Do any agency actions occur on tribal lands or ceded tribal lands? Use the following link to determine if tribal lands fall within the proposed action area:

a. <http://www.arcgis.com/home/webmap/viewer.html?webmap=2a19e6ffe6934e09aaa0fa82f1bc0148>

Yes – Contact the WS State Director and WS tribal liaison to determine if there is a need for formal consultation on the program/study. This study may not qualify for a Categorical Exclusion and **an EA or EIS may need to be prepared** before initiation of the project if there are any tribal concerns. (A copy of the tribal letter must be included when your study is archived). – Continue to question E.

No – Continue to question E.

**E.** Is the study a routine measures activity, such as identification, surveying, testing, removals, control, and sampling that will not cause physical alteration of the environment?

Yes – You must be able to check all the below boxes and provide justification (if you are unable to check all the boxes, you must check “No”) - Continue to question F.

1. Be localized or contained in areas where people are not likely to be exposed, and is limited in terms of quantity

2. Does not cause contaminants to enter water bodies (this includes runoff, drift or volatilization)

3. Does not cause bioaccumulation (the accumulation of a toxicant at a rate faster than it can be metabolized or excreted from an organism. In aquatic systems the bioconcentration factor (BCF) can be used to determine the potential for bioaccumulation. The octanol water partition coefficient (Kow) can also be used to determine the potential for bioaccumulation in aquatic and terrestrial organisms).

4. No extraordinary circumstances identified (adverse effects to environmentally sensitive areas or resources, or public controversy over the environmental effects of the proposed action)

Justification: Live trapping will be localized to one locations at a time. Trapping will occur in areas and times that will limit interactions with people, livestock, domestic and non-target wild animals. All traps will be removed at the end of the study and only non-lethal baits will be used (e.g. coconut meat). All non-target captures will be released immediately on site. Trap sites are near or along access roads or habitat edges with minimal impact to the surrounding vegetation.

No – Based on the information provided above this study does not qualify for a Categorical Exclusion and **an EA or EIS should be prepared** before initiation of the project. **You are done with this appendix. Contact the NEPA Coordinator for assistance.**

**F.** Summarize the risk to each group in the below with consideration of effects and the potential for exposure individually, and in relation to other impacts that may occur in the study area. Provide a justification for each endpoint and check the appropriate box below.

**Cumulative impacts** are impacts on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions. Cumulative impacts can result in individually minor but collectively significant actions taking place over a period of time.

1. Risk to human health
2. Risk to target species
3. Risk to non-target species

Justification:

1. There will be no risk to the human health and safety as the only environmental activities will be the capture of animals in live traps. No toxic baits or agents will be used..
2. No risk to the target species (target species are non-native and invasive and all individuals will be euthanized)
3. No risk to non-target species (trap type and placement will be optimized to exclude non-target captures which will be released on site)

Does this activity pose a risk to human health or target and non-target species (including cumulative impacts) that will not be minimized or mitigated?

Yes – Based on the information provided above this study does not qualify for a Categorical Exclusion and **an EA or EIS should be prepared** before initiation of the project. **You are done with this appendix. Contact the NEPA Coordinator for assistance.**

No – Continue to question G.

**G.** Will this study have a disproportionate adverse effect to children, minorities and low income populations? (Use the information under letter F (Risk to human health) and the location of the proposed study (i.e., potential for exposure) to discuss whether there would be any disproportionate impacts to children, minorities, and low income populations). [Click here to enter text](#)

Yes – Based on the information provided above this study does not qualify for a Categorical Exclusion and **an EA or EIS should be prepared** before initiation of the project. **You are done with this appendix. Contact the NEPA Coordinator for assistance.**

No – The study meets the criteria for Categorical Exclusion - **No further action is needed for NEPA.**