Case Study

The path to U.S. national registration of a toxic bait for the control of the small Indian mongoose

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Abstract: The small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]; mongoose) is a highly invasive species in its introduced range that negatively impacts ecosystems. Mongooses depredate native species, serve as a vector of disease posing a risk to human health, and cause sanitation issues in food processing facilities and public areas. Introduced for biocontrol in the late 1800s in Hawai'i and the Caribbean, mongooses currently have well-established populations across multiple islands in both island archipelagos and have invaded numerous other locations throughout the world. The concern of accidental introduction to mongoose-free islands, the difficulty in species detection, and the high cost and labor demand of trapping present the need for a novel control method. A target-specific and efficacious toxic bait can provide an additional tool to reduce mongoose abundance, to eradicate incipient populations, and for biocontrol at ports of entry. In this paper, we document the pathway to registration for a toxic bait for mongoose control with the U.S. Environmental Protection Agency. A registered product must demonstrate a low risk to nontarget species, meet standards for human health and safety, and show no unreasonable adverse effects to the environment. There are no other comparable invasive small mammalian carnivores for which toxic baits have been developed and registered for bait station deployment in the United States.

Key words: invasive species, registration, regulatory requirements, small Indian mongoose, toxicant, *Urva auropunctata*, vertebrate pesticide development

Small Indian mongooses (*Urva auropunctata* [syn. *Herpestes auropunctatus*]; mongoose) were widely introduced to Hawai'i, Puerto Rico, the U.S. Virgin Islands, continental areas in northeastern South America, Japan (Okinawa, Amami Oshima), and the Croatian peninsula, as well as numerous other locations throughout the world, primarily to control rodent pests and venomous snakes in tropical and subtropical agriculture areas (Figure 1). Mongooses are now considered among 100 of the world's most invasive species (Lowe et al. 2000, Hays and Conant 2007, Barun et al. 2011, Berentsen et al. 2018).

In the United States, mongooses have proliferated in natural areas throughout Hawai'i and have become well-established and difficult to control on the islands of Hawai'i, O'ahu, Maui, and Moloka'i, as well as islands in the Caribbean, including Puerto Rico and the U.S. Virgin Islands (Berentsen et al. 2018). Predation by mongooses has led to the decline and extirpation of native mammals, birds, reptiles, and amphibians (Nellis and Everard 1983, Yamada and Sugimura 2004, Hays and Conant

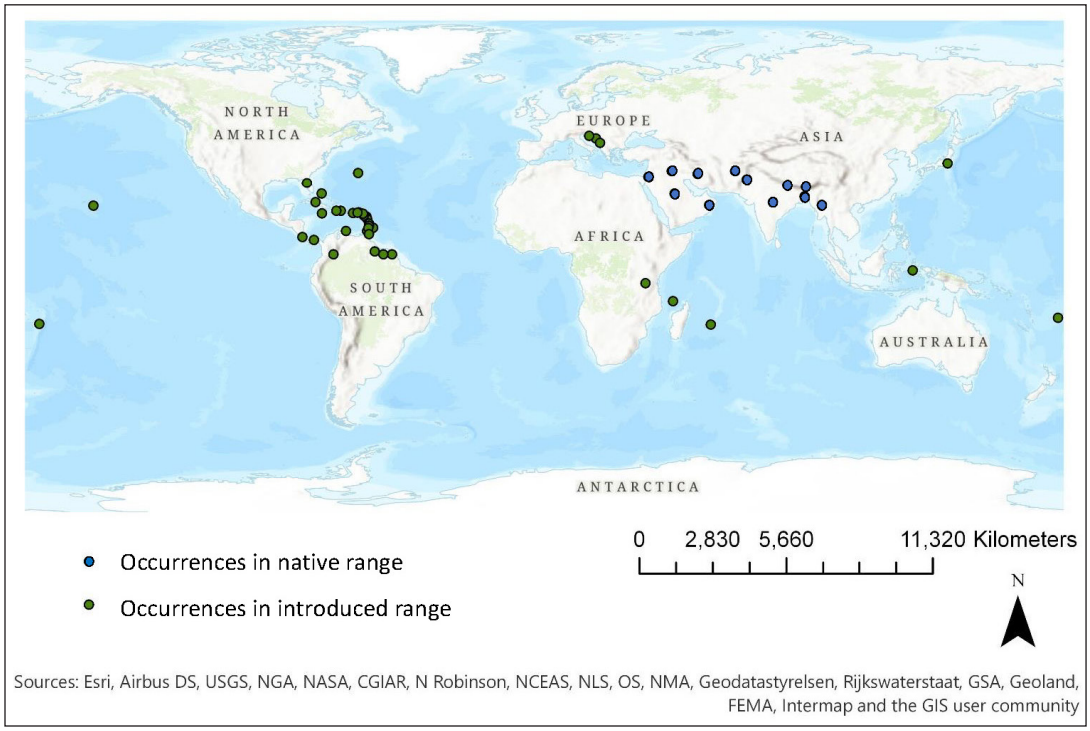

Figure 1. Global occurrences of the small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) within its introduced and native range. Data from the Centre for Agriculture and Bioscience International (2022).

2007). The small Indian mongoose is linked to the extinction of 9 native species in the Pacific and the Caribbean and is documented to threaten an additional 74 species found on the International Union for Conservation of Nature and Natural Resources Red List (Doherty et al. 2016). As predators of eggs and nestlings of native ground-nesting birds, mongooses continue to impede the recovery of native species globally (Banko 1992, Hays and Conant 2007, Yagihasi et al. 2021).

Mongooses also present a health risk to humans as hosts of leptospirosis in Hawai'i (Wong et al. 2012) and the Caribbean (Everard et al. 1976) and as a rabies reservoir on several islands in the Caribbean (Berentsen et al. 2018, Seetahal et al. 2018). The incursion of mongooses from adjacent orchards and waste areas into food packing, storage, and processing facilities as well as urban parklands presents sanitation and public health concerns.

Due to their invasive status and potential to spread disease, it is illegal to import, export, acquire, or transport mongooses under the Lacey Act of 1900 in the United States (18 U.S.C. § 42) and in Hawai'i under Hawai'i Administrative Rules 13-124-3. Nevertheless, accidental transportation continues to pose a risk of introduction to mongoose-free islands such as Kaua'i and Lāna'i in shipping containers, vehicles, construction materials, and possibly aircraft cargo (Tomich 1986, Menard et al. 2013, Berentsen et al. 2018).

Most recently, in May 2023, a gravid female mongoose was trapped at Nāwiliwili Harbor, Kaua'i, after a reported sighting (Hawai'i Department of Agriculture [HDOA]). In December 2021, another live mongoose was caught at Nāwiliwili Harbor, Kaua'i (HDOA; Figure 2). In 2018, a mummified mongoose carcass was found in a truck engine compartment in Līhu'e, Kaua'i (HDOA, Wildlife Services [WS]-Kaua'i; Figure 3). Other incursion events on Kaua'i include a lactating female found dead in 1976 along a road near the town of 'Ele'ele and 2 mongooses that were captured in 2012—one at Nāwiliwili Harbor and the other near a resort in Līhu'e (Kaua'i Invasive Species Committee [KISC] 2016; Figure 4). Another mongoose was captured at Līhu'e Airport, Kaua'i, in October

Figure 2. Small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) live capture at Nāwiliwili Harbor, Kauaʻi, Hawaiʻi, USA, in 2021 (*photo courtesy of the Hawaiʻi Department of Agriculture*).

Figure 3. Small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) carcass found in a car engine compartment on Līhuʻe, Kauaʻi, Hawaiʻi, USA, in 2018 (*photo courtesy of the Hawaiʻi Department of Agriculture and the U.S. Department of Agriculture Wildlife Services-Kauaʻi*).

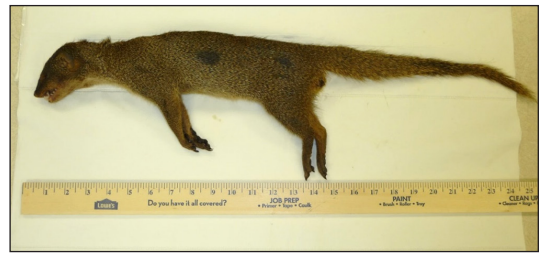

Figure 4. Small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) carcass from live capture on Kauaʻi, Hawaiʻi, USA, in 2012 (*photo courtesy of the Kauaʻi Invasive Species Committee*).

Figure 5. Small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) live capture at Aloha Air Cargo, Līhuʻe Airport, Līhuʻe, Kauaʻi, Hawaiʻi, USA, in 2016 (*photo courtesy of the Hawaiʻi Department of Agriculture*).

2016 (KISC 2016; Figure 5). Although there have been many reported sightings on Kauaʻi, only small numbers have been confirmed, and there is no evidence of an established population on the island (Berentsen et al. 2018). Managers have aggressively tracked reported mongoose sightings on Kauaʻi, including the development of adaptive early detection and rapid response procedures (Phillips et al. 2016), emphasizing the urgent need for effective methods to intercept any new arrivals and prevent the establishment of new populations in mongoose-free ecosystems.

Trapping is used almost exclusively and has been useful in reducing mongoose populations and predation in and around targeted sensitive native species such as ground-nesting upland birds and seabird colonies, but has limitations. Trapping is labor-intensive, expensive, and only removes individuals from limited areas (Barun et al. 2011, Berentsen et al. 2018, Sugihara et al. 2018). Toxic baits and other toxicant delivery systems can provide a more effective and longer-lasting approach to control and/or eradicate mongooses from larger areas and may be used as an alternative method to intercept small numbers of accidentally introduced individuals in new locations where they may be difficult to detect and trap. Live traps may require multiple checks each day by personnel while toxicants may only require checks once or twice each week. Currently, there is limited use of toxicants for controlling mongooses due to the lack of available and efficacious registered

products. Furthermore, there are no other comparable small mammalian carnivores for which toxic baits have been developed and registered in the United States. Our objective in this paper is to present a case study documenting the pathway to registration for a toxic bait for mongoose control with the U.S. Environmental Protection Agency (EPA).

History of registered toxicants for mongooses

In the United States, toxic baits for vertebrate pest control can either be registered by the EPA as a pesticide product under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA; Public Law No. 61–152, 7 U.S.C. § 136) or by an individual state under Section 24(c), which are also called Special Local Needs (SLN) registrations. Previously, 3 SLN products used in bait stations were registered in Hawai'i for mongooses. All SLN registrations contained the active ingredient diphacinone, a "first generation" anticoagulant toxicant. Mongooses are extremely susceptible to diphacinone, with a median lethal dose (LD_{50}) of 0.18 mg/kg body weight (Keith et al. 1989).

The first SLN registration, in 1991, was a diphacinone concentrate product that was mixed with raw ground beef (0.00025% diphacinone, SLN Reg. No. HI-910004, EPA Reg. No. 12455-9; Keith et al. 1989). The ground beef matrix was highly palatable to mongooses but required pre-mixing diphacinone with raw ground beef and frequent bait replacement in the field due to rapid spoilage. The SLN registration was canceled in 1996, mainly due to limited use (Sugihara et al. 2018).

The second SLN registration, in 1997, was for "Eaton's® All Weather Bait Blocks® Rodenticide with Fish Flavorizer™" (0.005% diphacinone, SLN Reg. No. HI-970007, EPA Reg. No. 56-44; J.T. Eaton & Co. Inc., Twinsburg, Ohio, USA), labeled for use on mongooses and invasive rodents. This product appeared to be efficacious for mongooses (Smith et al. 2000), but the registration was eventually canceled as well, likely due to rapid deterioration in the warm and humid environment in Hawai'i and concerns of viable exotic plant seeds in the bait matrix (R. T. Sugihara, WS-Hawai'i Field Station, personal communication).

The third product received SLN registration in 1998 and remains the only registered toxic bait available for mongoose control. This product is a hard, waxy, grain-based bait block named "Ramik® Mini Bars Kills Rats and Mice" (0.005% diphacinone, SLN Reg. No. HI-980005, EPA Reg. No. 61282-26; HACCO, Inc., Randolph, Wisconsin, USA) and is labeled for use on mongooses and invasive rodents in conservation areas. The sale of this SLN product is restricted to federal and state wildlife management agencies for use only under the direct supervision of certified pesticide applicators with prior project approval from the U.S. Fish and Wildlife Service (USFWS; https://files.hawaii.gov/hdoa/labels/sln/9805_2024.pdf). This bait has varying reports of success for controlling mongooses (Young et al. 2013, VanderWerf and Young 2014). Poor bait acceptance and palatability of the hard cereal-bait by mongooses are suspected to limit its efficacy (R. T. Sugihara, personal communication).

Furthermore, in addition to the first SLN products registered in Hawai'i, diphacinone baits have been used to control mongoose populations in other parts of the world. In cooperation with Japanese researchers attempting to control mongooses on Okinawa and Amami-Oshima, Japan, an encased sausage bait containing 0.005% diphacinone was found to be equally efficacious for mongooses in laboratory cage and field enclosure trials conducted in Okinawa (R. T. Sugihara, 2016 and 2018 Japan trip reports). Subsequent experimental field trials with diphacinone mixed with minced chicken were conducted on Amami-Oshima in isolated locations along steep terrain where trapping was not feasible. Preliminary results showed that the diphacinone bait was successful in eliminating the remnant mongoose population from the baited areas (T. Jogahara, Okinawa University, personal communication). This demonstrated the potential for optimizing the susceptibility of mongooses to diphacinone in another more palatable bait matrix with better field longevity (Sugihara et al. 2018).

The road to U.S. national registration

To register a vertebrate toxic bait for use across multiple states and territories in the United States requires a national (Section 3) pesticide registration with the EPA. Although

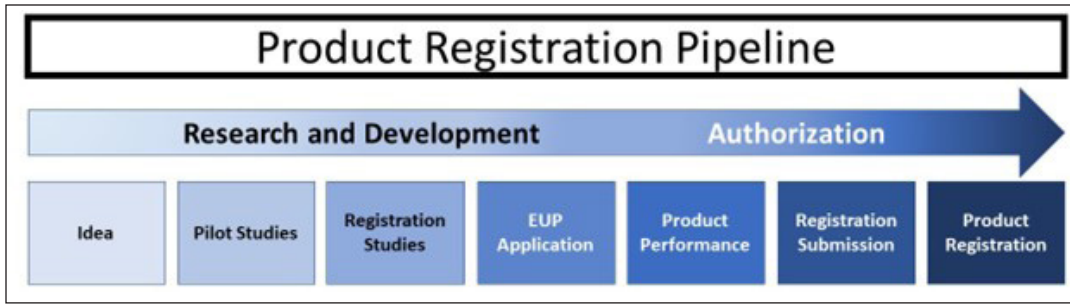

Figure 6. Product pipeline for vertebrate pesticide registration with the U.S. Environmental Protection Agency.

a vertebrate toxic bait has not yet been registered for a comparable small mammalian carnivore, the registration process is modeled after rodent toxic bait development (Figure 6). The product must first be shown to be sufficiently efficacious and palatable to mongooses in the laboratory in accordance with the EPA OPPTS 810.1000 guideline in order to support the issuance of an Experimental Use Permit (EUP) for a larger field product performance study.

The EUP application must also include a subset of the registration data required by the EPA for a full Section 3 registration of this type of toxic bait and proposed use pattern, including product chemistry, toxicology, ecological effects, and environmental fate studies on the active ingredient and/or final bait formulation (Ruell et al. 2019). These registration data submissions must meet EPA study requirements (40 C.F.R. § 158.70) and be conducted under FIFRA Good Laboratory Practices (GLP) standards (40 C.F.R. § 160). The full set of registration data required for a subsequent Section 3 registration application includes the product performance (EUP field) study, and any remaining product chemistry, toxicology, ecological effects, and environmental fate studies.

Compliance with the EPA's FIFRA GLP standards is required for all studies supporting any pesticide registration in the United States (40 C.F.R. § 160.1). These GLP requirements include, but are not limited to, requirements for study protocols, study personnel, testing facilities, standard operating procedures, equipment, data entry and archiving, final reports, and a Quality Assurance Unit that monitors and audits the study. Research studies with animals also must be conducted in compliance with the Animal Welfare Act standards for animal care and require prior approval from an Institution Animal Care and Use Committee.

Methods

We summarize the chronology of the steps the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center (NWRC) has made toward the registration of a new toxic bait product for mongooses. The studies we document were conducted at the NWRC Hawai'i Field Station over a 5-year period with support from the bait manufacturer, the NWRC Product Registration Unit, and the NWRC Chemistry Laboratory Unit. Financial support was provided by the Hawai'i Invasive Species Council and the USFWS Ecological Services Office.

To aid in the registration process, the NWRC's Technology Transfer Program facilitates product registrations through Confidentiality Agreements, Material Transfer Research Agreements, Cooperative Research and Development Agreements (CRADAs), patents, and licensing. In the case of the mongoose toxic bait, a Material Transfer Research Agreement, CRADA, and USDA Veterinary Services Permit to import and/or transport-controlled materials were required for the collaboration between the NWRC and the international bait manufacturer.

Screening toxicants

In 5-day no-choice laboratory feeding trials (no nontoxic alternative diet available), 10 commercially registered rodenticide bait products with 7 active ingredients and 2 new candidate acute active ingredients were evaluated for palatability and toxicity against mongooses as an initial step to determine potential active ingredients and bait matrices for further

Table 1. Mean bait consumption [g], mean overall toxin consumed [mg/kg], and mortality [%] for mongooses (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) fed commercial and experimental toxicants. Adapted from Sugihara et al. (2018).

Bait type	Dosage	Mean overall bait consumption [%], 50 g offered daily		Mean overall toxin consumed [mg/kg]		% Mortality (N = 10)
		Male	Female	Male	Female	
Ramik® Mini Bars Kills Rats and Mice (blocks)	0.005% diphacinone	3.61	0.81	0.32	0.05	20
Ramik® Green (pellets)	0.005% diphacinone	11.40	5.34	0.96	0.35	50
Rozol® (pellets)	0.005% chlorophacinone	0.19	3.70	0.02	0.20	20
Brodifacoum 25W® Conservation (pellets)	0.0025% brodifacoum	2.11	6.12	0.11	0.15	40
Resolv® (soft bait)	0.005% bromadiolone	30.36	20.32	2.17	0.83	30
Boot Hill® (pellets)	0.005% bromadiolone	0.18	0.29	0.01	0.01	10
Fast Draw® (soft bait)	0.0025% difethialone	34.08	24.42	0.93	0.42	50
Tomcat® Mouse and Rat Killer (blocks)—trial 1	0.01% bromethalin	26.36	26.64	0.84	0.82	100
Tomcat® Mouse and Rat Killer (blocks)—trial 2	0.01% bromethalin	11.46	13.2	0.69	0.38	90
Terad$_3$® (blocks)	0.075% cholecalciferol	6.28	6.35	9.93	6.94	50
Terad$_3$® (pellets)	0.075% cholecalciferol	2.46	1.43	2.82	1.15	20
Diphacinone with minced chicken	0.005% diphacinone	100	100	12.07	17.87	100
Para-aminopropiophenone (PAPP) with minced chicken	0.15% PAPP	65.18	54.19	86.05	110.41	100
Sodium nitrite (SN) with minced chicken	5.0% SN	32.43	29.39	1,206.48	1,718.82	30

mongoose toxic bait development (Sugihara et al. 2018; Table 1). As there are no similar products registered in the United States for small carnivores, rodent products were used instead because rodents can be managed with the same active ingredients and co-labeled products (Table 2). Acceptance and palatability were poor for 9 of the 10 commercial rodenticides (hard grain-based pellets and block baits, and soft baits) with subsequent low (10–50%) overall mortality. The exception was Tomcat® Rat and Mouse Killer (Bell Laboratories, Inc., Madison, Wisconsin), a hard bait block containing 0.01% bromethalin, which is an acute neurotoxin. A symptom of bromethalin toxicosis is appetite suppression; although mongooses only ate an average of 19% of the bait offered, 95% succumbed across two trials (Sugihara et al. 2018).

The diphacinone bait currently registered for rat and mongoose control in Hawai'i, "Ramik Mini Bars Kills Rats and Mice," achieved only 20% mortality in laboratory trials (Sugihara et al. 2018). Suspecting low palatability of the hard cereal bait matrix (mean consumption of bait offered was only 3.61% for males and 0.81% for females), 0.005% technical diphacinone in fresh minced chicken was trialed and achieved 100% mortality over a 3-day no-choice feeding trial (Sugihara et al. 2018). Unsurprisingly, mongooses as carnivores were more at-

Table 2. Types of vertebrate toxicant compounds considered for control of the invasive small Indian mongoose (*Urva auropunctata* [syn. *Herpestes auropunctatus*]).

Compound	Mode of action	Other human and animal uses	U.S. registrations
Para-aminopropiophenone (PAPP)	Methemoglobinemia	Human drug, predacide	None
Sodium nitrite (SN)	Methemoglobinemia	Food additive, human drug (cyanide poisoning antidote), insecticide, predacide	None
Bromethalin	Neurotoxin	Rodenticide	Rodenticide
Diphacinone	Anticoagulant	Anticonvulsant drug, rodenticide	Rodenticide
Chlorophacinone	Anticoagulant	Rodenticide	Rodenticide
Brodifacoum	Anticoagulant	Rodenticide	Rodenticide
Bromadiolone	Anticoagulant	Rodenticide	Rodenticide
Difethialone	Anticoagulant	Rodenticide	Rodenticide
Cholecalciferol	Hypercalcemia	Dietary (Vitamin D) supplement, human drug, rodenticide	Rodenticide

tracted to soft, meat-based diphacinone bait.

Single-day feeding of microencapsulated para-aminopropiophenone (PAPP), a chemical that reduces the oxygen-carrying capacity of the blood, achieved 100% mortality at the concentration of 0.15% in minced chicken (Sugihara et al. 2018). Microencapsulated sodium nitrite (SN), which has a similar toxic mode of action as PAPP, was formulated in minced chicken at 5% but was poorly accepted by mongooses with an average mortality of 30% (sodium nitrite is extremely salty and desiccated the bait when microencapsulation broke down). Sugihara et al. (2018) determined that diphacinone, bromethalin, and PAPP formulated in a more palatable bait would be potential candidate toxicants to pursue for mongoose control.

Subsequently, Ruell et al. (2019) conducted a registration feasibility analysis and risk assessment for the 4 most likely candidate active ingredients: PAPP, SN, bromethalin, and diphacinone, which compared likely registration costs and timelines, humaneness, antidote availability, and convenience-of-use for each active ingredient. The SN was included because palatability could potentially be improved in a more compatible bait matrix or lower concentrations and it had other potentially advantageous characteristics. For a "bait station only" product for mongooses, the paths toward registering PAPP and SN baits were found to be relatively slow and more expensive to register because of the lack of previously registered products in the United States. In contrast, a bromethalin or diphacinone bait would be the least expensive and fastest to register, especially given that numerous registered formulations are already commercially available for both active ingredients. The only bromethalin product tested proved efficacious for mongooses (Sugihara et al. 2018). However, bromethalin was ranked lower than PAPP or SN for overall humaneness and had a higher risk of direct acute toxicity to nontarget species than diphacinone. Of the 4, diphacinone had the lowest risk to nontarget species because it requires multiple feedings to be fatal for most species, which not only means it is less acutely toxic at the concentration used in registered toxic baits, but also that a nontarget individual's probability of exposure to a lethal dose in the field is reduced because they have to encounter and consume sufficient quantities of the toxic bait multiple times rather than just once. Diphacinone also has an antidote (vitamin K) that is approved by the U.S. Food and Drug Administration, easily accessible, and efficacious, and has a relatively slow mechanism of action providing time to administer the antidote in the event of an accidental exposure (Ruell et al. 2019). Additionally, if

Table 3. Mean bait consumption (g) by mongooses (*Urva auropunctata* [syn. *Herpestes auropunctatus*]) fed commercial nontoxic bait matrices. Adapted from Siers et al. (2020).

Bait matrix	Bait type	Mean daily consumption (g)
FOXECUTE®	Semi-soft meat-based bait	24 g ± 13.01 SD
FOXSHIELD®	Semi-soft fish-based bait	22 g ± 8.63 SD
HOGGONE®	Peanut paste-based bait	15 g ± 7.40 SD
"Brown treesnake bait"	Semi-soft pork-based bait	31 g ± 11.75 SD

alternative application methods outside of bait stations will be required for mongooses in the future, diphacinone is already registered for broadcast and other application methods for island conservation areas. Ancillary registration data are already available to support these application methods for diphacinone (Ruell et al. 2019).

Screening bait matrices

The development of an effective mongoose bait product requires a soft, palatable, more durable bait matrix that is longer lasting and easier to use in the field than fresh raw meat. Based on previous laboratory trials, mongooses preferred raw meat matrices over hard bait blocks (Sugihara et al. 2018), but raw meat degrades rapidly in the field. A softer bait matrix formulated to attract carnivores and that persists in the field under field conditions will likely be effective for a mongoose toxic bait matrix. Preliminary 2-choice feeding trials (bait offered alongside a nontoxic alternative challenge diet) evaluated the palatability of 4 candidate nontoxic soft bait matrices for mongooses to determine which had adequate palatability and sufficient consumption to warrant future consideration as a bait matrix for diphacinone (Siers et al. 2020). The 4 candidate nontoxic bait matrices included 2 nontoxic versions of baits registered for fox (*Vulpes vulpes*) control in Australia and produced by Animal Control Technologies, Australia (ACTA; EPA Establishment No.: 091731-AUS-001): FOXSHIELD®, a fish-based solid cylindrical bait, and FOXECUTE®, a meat-based solid cylindrical bait. The third was a nontoxic version of HOGGONE® (ACTA), semi-solid peanut paste-based bait registered in Australia for feral swine (*Sus scrofa*) control. The fourth was a nontoxic version of the "brown treesnake bait," a processed pork shoulder loaf formulated with synthetic lipids mimicking the scent profile of dead mice that is under development by NWRC for invasive brown treesnake (*Boiga irregularis*) control (Kimball et al. 2016, Garcia et al. 2021). All bait types had high daily average consumption (15–31 g per mongoose) and were preferred over the dry dog (*Canis familiaris*) kibble challenge diet (Siers et al. 2020; Table 3). The "brown treesnake bait" had the highest daily average consumption (31 g; Table 3) but was not selected for further investigation because it is not commercially available and spoils more quickly due to moisture content.

The most promising candidate bait matrix was the nontoxic ACTA fish-based bait, which is a preserved, semi-soft, fish-based cylinder bait encased in a sausage-type skin. This bait is formed into easy-to-handle discrete bait pieces and had high daily consumption (22 g ± 8.63) (Siers et al. 2020; Table 3). Additionally, the nontoxic ACTA fish-based bait matrix already has a commercial pesticide manufacturer that can produce multiple batches of bait with long storage and field longevity. Fish-based products have traditionally been very attractive to mongooses and have fewer importation requirements compared to meat-based products (Siers et al. 2020).

Laboratory efficacy testing of the bait matrix and active ingredient

Based on the previous evaluations of the candidate active ingredients and nontoxic bait matrices, diphacinone at the standard concentration used in rodent baits for field use (0.005%) and the ACTA fish-based bait matrix were selected for further laboratory efficacy trials (U.S. Environmental Protection Agency 2015, Siers et al. 2020). Because there are no EPA study guidelines for testing toxic baits for mongooses, the study design and protocol were modified from the EPA Office of Pesticide Programs guideline 1.203 for dry anticoagulant products for rodents. The EPA provided their review and comments on the proposed protocol in Septem-

Figure 7. Two pieces of the preserved, cylindrical, semi-soft, fish-based diphacinone bait product produced by Animal Control Technologies, Australia (U.S. Environmental Protection Agency Establishment No.: 091731-AUS-001).

ber 2020, and additional minor modifications were made to the protocol to address these comments, including the selection of challenge and maintenance diets that were closer in texture to the candidate toxic bait.

A supplementary test evaluated the incorporation process of achieving uniform dispersal of diphacinone into a potential product and confirmed the production scaleup process for the chosen method. Bait uptake was sufficient for 100% lethality (6/6 mongooses) in a 5-day 2-choice study (Sugihara et al. 2021*a*).

Based on these promising preliminary results, a larger 2-choice, GLP laboratory efficacy study on the proprietary ACTA fish-based diphacinone bait product was conducted (Sugihara et al. 2021*b*; Figure 7). There was 85% mortality (17/20 mongooses) in the treated group during the 5-day 2-choice test and 15-day post-test periods (Sugihara et al. 2021*b*). The fish-based bait with diphacinone appeared to be sufficiently palatable and efficacious after a 5-day exposure for wild-caught mongooses. This trial was reviewed by the EPA during their review of the submitted EUP application for a field product performance study. The EUP was approved by EPA and then the Hawai'i Department of Agriculture in 2023. The field study is scheduled to begin in 2024.

Bait station delivery system

The initial EPA registration for the upcoming mongoose toxic bait will be limited to bait stations for terrestrial non-crop use to prevent nontarget take of the toxic bait. Bait take by nontarget species can jeopardize the health of native species, and rapid consumption of bait by rats and other consumers would make the bait unavailable for mongooses that forage over large areas, especially within low-density populations. We aimed to design an effective bait station that targets mongooses, reduces consumption by rats (the primary bait co-consumer), and excludes other nontarget species including native birds.

To develop a mongoose bait station, candidate bait station designs for mongooses were trialed in laboratory and field studies using the nontoxic ACTA fish-based bait matrix (Antaky et al. 2023). Modified versions of the PVC tube inverted "T" bait station (Keith et al. 1989), commercially available sturdy plastic rodenticide bait stations, and novel prototype designs were evaluated. Bait station designs were tested in enclosed arenas and monitored with video cameras. These bait stations were further evaluated in the field with free-ranging mongooses and other species that may also visit bait stations and monitored with motion detection cameras. Bait was monitored for weathering, spoilage, and consumption by insects, mollusks, and other detritivores in the field. The most practical bait station design that performed well with high mongoose bait consumption and low nontarget bait interaction and consumption will be selected for the EUP field study. Deployment modifications may be needed, and the use of bait stations may be curtailed in habitats occupied by feral swine or other large mammals that might destroy or interfere with the bait stations.

Additional registration data for the Experimental Use Permit application

In addition to the GLP laboratory efficacy data, the EPA required that "Group A" product chemistry registration data, which includes composition, production, and formulation of the active and inert ingredients, and a subset of the "Group B" product chemistry registration data, which includes additional physical and chemical properties of the product, described in Ruell et al. (2019), were submitted in the EUP application. These studies were conducted on the final bait formulation by the NWRC Chemistry and Registration Units or by contract laboratories. These product chemistry stud-

ies include a description and evaluation of the bait's composition, physical and chemical characteristics, and 5-batch manufacture consistency. Existing product chemistry, toxicology, ecological effects, and environmental fate registration data were cited for the registered source of the active ingredient, diphacinone. Usually, if a bait formulation has never been registered before, the toxicology "6-pack" registration studies are required on the final formulation itself. These studies provide acute oral, acute dermal, acute inhalation, eye irritation, skin irritation, and skin sensitization data. However, when a formulation is similar in composition and concentration of the active ingredient to already-registered products, which is the case for diphacinone active ingredient, the 6-pack toxicology data from these similar products will be cited in lieu of generating new data as the human health risks posed by the products will be similar.

The Consolidated Appropriations Act of 2004 (e.g., Pesticide Registration Improvement Act or PRIA) created a new system for EPA pesticide registration and requires the EPA to review applications within set decision times and has been reauthorized by Congress multiple times. The current reauthorization, the Pesticide Registration Improvement Extension Act of 2022 (also known as PRIA 5; Division HH, Title VI of P.L. 117-328) sets the EPA's review time for an EUP for this type of product at 6 months (Ruell et al. 2019; updated for PRIA 5).

Product performance (field) trials under an EPA experiment use permit

The EUP field efficacy trials will be performed using the selected and tested fish-based diphacinone bait and bait station design. The EUP protocol approved by the EPA will dictate the exact methodological procedures for field trials for data needed to support the forthcoming registration of the mongoose toxic bait. The product used in the EUP must be the same formulation intended for the final registration.

Field trials of the mongoose toxic bait under the EUP will be conducted in different environments representative of the proposed use patterns. These sites will also be selected to have strong differences in humidity to be typical of conditions where the toxic bait could be used in other locations in the United States such as the U.S. territories in the Caribbean Islands. Nontarget species visiting bait stations will be identified in camera images, and any bait consumption or removal that can be observed on images will be recorded. Additionally, free-ranging mongooses within the treatment plots will be individually monitored using radio telemetry to measure mortality during the study.

All nontarget carcasses found within the study area will be examined for evidence of diphacinone toxicity. Diphacinone residues in rodents are potentially a concern because they may be consumed by predators such as the Hawaiian hawk (*Buteo solitarius*) and Hawaiian short-eared owl (*Asio flammeus sandwichensis*). Free-ranging chickens (*Gallus gallus* spp.), which may be common in these study areas, will be representative of agricultural situations. Mongoose carcasses collected within the study area during and after the trial will also be examined for evidence of diphacinone toxicity to confirm the cause of death.

Section 3 registration

The final step to register a vertebrate pesticide product for national use in the United States is to submit a Section 3 registration application to the EPA. The future Section 3 registration application for the ACTA fish-based diphacinone bait for mongooses will include submission of the proposed commercial pesticide label, a Confidential Statement of Formula, the GLP product performance (EUP field) study, a 1-year storage stability study on the bait, and any additional GLP registration studies identified by the EPA during their review of the EUP application.

The EPA's statutory review time for this Section 3 registration application under PRIA 5 will be between 12 and 15 months, depending on how the product is classified during EPA front desk screening (Ruell et al. 2019; Table 1, updated for PRIA 5). The EPA can only approve applications if the vertebrate pesticide product meets EPA guidelines and is found to "not cause unreasonable adverse effects on human health or the environment when using according to the label," as stated in the FIFRA. Following federal registration, the product must also be registered in each state and U.S. territory where it will be used before distribution and operational use.

Discussion

The development of an effective mongoose toxic bait for use as a conservation tool in the United States would benefit native wildlife and human health and provide another means to prevent the establishment of mongooses on mongoose-free islands. The toxic bait and delivery system should demonstrate a high level of efficacy and acceptable risks with respect to nontarget species. Studies supporting this development will be subject to scrutiny by regulatory authorities in their evaluation of the risks to humans as well as to nontarget species for the product's proposed use pattern to control mongooses in Hawai'i and other locations in the United States. This or similar products also have potential application in other countries (Yagihashi et al. 2021).

This case study outlines the development pathway of a novel vertebrate toxic bait registration and continues the momentum toward the eventual goal of field deployment of an effective toxic bait for mongoose control in agriculture, biosecurity, and conservation applications. This work also demonstrates effective collaboration between public and private sector research groups across international boundaries, which has accelerated progress toward a practical and effective outcome. The EUP application was approved by the EPA (EUP No.: 56228-EUP-45) in April of 2023. Due to study length requirements to meet EPA standards within the remaining required registration studies and EPA review times, we anticipate a timeline of 2–3 years to securing a forthcoming registration if the development research remains funded and prioritized. Once the initial product for use in bait stations is registered, ongoing data may support the eventual registration of additional use outside of bait stations for larger scale mongoose control and/or eradications on islands for conservation purposes.

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Literature cited

Antaky, C. C., R. T. Sugihara, I. L. Leinbach, S. R. Siers, E. W. Ruell, and S. C. Hess. 2023. Development and evaluation of prototype toxicant-delivery bait stations for the control of the small Indian mongoose. Management of Biological Invasions 14:775–791.

Banko, P. C. 1992. Constraints on the productivity of wild nene or Hawaiian geese *Branta sandvicensis*. Wildfowl 43:99–106.

Barun, A., C. C. Hanson, K. J. Campbell, and D. Simberloff. 2011. A review of small Indian mongoose management and eradications on islands. Pages 17–25 *in* C. R. Veitch, M. N. Clout, and D. R. Towns, editors. Island invasives: eradication and management. International Union for Conservation of Nature, Gland, Switzerland.

Berentsen, A. R., W. C. Pitt, and R. T. Sugihara. 2018. Ecology of the small Indian mongoose (*Herpestes auropunctatus*) in North America. Pages 251–267 *in* W. C. Pitt, J. C. Beasley, and G. W. Witmer, editors. Ecology and management of terrestrial vertebrate invasive species in the United States. CRC Press, Boca Raton, Florida, USA.

Centre for Agriculture and Bioscience International. 2022. *Herpestes auropunctatus* (small Indian mongoose). Invasive Species Compendium, Centre for Agriculture and Bioscience International, Wallingford, United Kingdom, <https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.80508>. Accessed January 2, 2023.

Doherty, T. S., A. S. Glen, D. G. Nimmo, E. G. Ritchie, and C. R. Dickman. 2016. Invasive predators and global biodiversity loss. Proceedings of the National Academy of Sciences 113(40):11261–11265.

Everard, C. O. R., A. E. Green, and J. W. Glosser. 1976. Leptospirosis in Trinidad and Grenada,

with special reference to the mongoose. Royal Society of Tropical Medicine and Hygiene 70:57–61.

Garcia, R. A., T. W. McAuliffe, L. P. Bumanlag, S. R. Siers, and B. A. Kimball. 2021. Adaptation of an artificial bait to an automated aerial delivery system for landscape-scale brown treesnake suppression. Biological Invasions 23:3175–3185.

Hays, W. S. T., and S. Conant. 2007. Biology and impacts of Pacific island invasive species. 1. A worldwide review of effects of the small Indian mongoose, *Herpestes javanicus* (Carnivora: Herpestidae). Pacific Science 61:3–16.

Kaua'i Invasive Species Committee (KISC). 2016. "Live mongoose captured on Kauai!" Kaua'i Invasive Species Committee blog. October 11, 2016. Kaua'i Invasive Species Committee, Kapa'a, Hawai'i, USA, <kauaiisc.org/live-mongoose-captured-on-kauai/>. Accessed September 10, 2021.

Keith, J. O., D. N. Hirata, D. L. Epsy, S. Greiner, and D. Griffin. 1989. Field evaluation of 0.00025% diphacinone bait for mongoose control in Hawaii. Denver Wildlife Research Center, Denver, Colorado, USA.

Kimball, B. A., S. A. Stelting, T. W. McAuliffe, R. S. Stahl, R. A. Garcia, and W. C. Pitt. 2016. Development of artificial bait for brown treesnake suppression. Biological Invasions 18:359–369.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. The Invasive Species Specialist Group of the Species Survival Commission of the World Conservation Union. First published in *Aliens* 12, December 2000. Reprinted November 2004. Hollands Printing, Auckland, New Zealand.

Menard, T., K. Gundersen, and J. Chapman. 2013. Kaua'i mongoose: can we eradicate them? Abstract. Hawai'i Conservation Conference, Honolulu, Hawai'i, USA, <https://www.hawaiiconservation.org/wp-content/uploads/final_2013_hcc_abstract_book.pdf>. Accessed September 15, 2021.

Nellis, D. W., and C. O. R. Everard. 1983. The biology of the mongoose in the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands 64:1–162.

Phillips, R. B., B. Lucey, T. Bogardus, W. Pitt, R. Sugihara, M. Takahiro, F. Yamada, M. Clark, M. Fry, and A. Nadig. 2016. Kauai mongoose standard operating procedures to conduct an islandwide status assessment and early detection rapid response. Kaua'i Invasive Species Committee, Kapa'a, Hawai'i, USA, <https://www.kauaiisc.org/wp-content/uploads/Kauai-Mongoose-SOPs-for-Island-Wide-Surveys-Early-Detection-Rapid-Response-2016-1.pdf>. Accessed September 13, 2021.

Ruell, E. W., C. N. Niebuhr, R. T. Sugihara, and S. R. Siers. 2019. An evaluation of the registration and use prospects for four candidate toxicants for controlling invasive mongooses (*Herpestes javanicus auropunctatus*). Management of Biological Invasions 10:573–596.

Seetahal, J. F. R., A. Vokaty, M. A. N. Vigilato. C. V. F. Carrington, J. Pradel, B. Louison, A. V. Sauers, R. Roopnarine, J. C. G. Arrebato, M. F. Millien, C. James, and C. E. Rupprecht. 2018. Rabies in the Caribbean: a situational analysis and historic review. Tropical Medicine and Infectious Diseases 3:89.

Siers, S. R., R. T. Sugihara, I. L. Leinbach, D. Sedgwick, C. N. Niebuhr, and E. W. Ruell. 2020. Development and testing of a matrix for mongoose toxic bait: nontoxic bait acceptance cage trials. Proceedings of the Vertebrate Pest Conference 29:1–7.

Smith, D. G., J. T. Polhemus, and E. A. VanderWerf. 2000. Efficacy of fish-flavored diphacinone bait blocks for controlling small Indian mongoose (*Herpestes auropunctatus*) populations in Hawai'i. 'Elepaio 60:47–51.

Sugihara, R. T., C. C. Antaky, and I. L. Leinbach. 2021*a*. Preliminary laboratory evaluation of the palatability of two experimental fish-based diphacinone (0.005%) baits for mongooses. Report QA-3298, National Wildlife Research Center, Hawai'i Field Station, Hilo, Hawai'i, USA.

Sugihara, R. T., C. C. Antaky, and I. L. Leinbach. 2021*b*. Two-choice laboratory efficacy test in mongooses—fish-based bait for mongooses (0.005% diphacinone). Report QA-2834, National Wildlife Research Center, Hawai'i Field Station, Hilo, Hawai'i, USA.

Sugihara, R. T., W. C. Pitt, A. R. Berentsen, and C. G. Payne. 2018. Evaluation of the palatability and toxicity of candidate baits and toxicants for mongooses (*Herpestes auropunctatus*). European Journal of Wildlife Research 64:2.

Tomich, P. Q. 1986. Mammals in Hawaii. Second edition. Bishop Museum Special Publication 76. Bishop Museum Press, Honolulu, Hawai'i, USA.

U.S. Environmental Protection Agency. 2015. Memorandum. Registration review—preliminary problem formulation for ecological risk and environmental fate, endangered species, and drinking water assessments for diphacinone and diphacinone sodium salt. Document USEPA-HQ-OPP-2015-0777-0005, U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C., USA, <https://www.regulations.gov/document?D=EPA-HQ-OPP-2015-0777-0005>. Accessed September 14, 2021.

VanderWerf, E. A., and L. C. Young. 2014. Breeding biology of red-tailed tropicbirds *Phaethon rubricauda* and response to predator control on O'ahu, Hawai'i. Marine Ornithology 42:73–76.

Wong, M., A. R. Katz, D. Li, and B. A. Wilcox. 2012. Leptospira infection prevalence in small mammal host populations on three Hawaiian Islands. American Journal of Tropical Medicine and Hygiene 87:337–341.

Yagihashi, T., S. I. Seki, T. Nakaya, K. Nakata, and N. Kotaka. 2021. Eradication of the mongoose is crucial for the conservation of three endemic bird species in Yambaru, Okinawa Island, Japan. Biological Invasions 23:2249–2260.

Yamada, F., and K. Sugimura. 2004. Negative impact of an invasive small Indian mongoose *Herpestes javanicus* on native wildlife species and evaluation of a control project in Amami-Oshima and Okinawa Islands, Japan. Global Environmental Research 8:117–124.

Young, L. C., E. A. VanderWerf, M. T. Lohr, C. J. Miller, A. J. Titmus, D. Peters, and L. Wilson. 2013. Multi-species predator eradication within a predator-proof fence at Ka'ena Point, Hawai'i. Biological Invasions 15:2627–2638.

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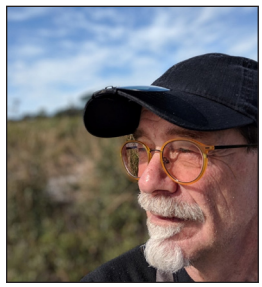

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