TESTING THE DERMAL AND ORAL TOXICITY OF SELECTED CHEMICALS TO BROWN TREESNAKES

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ABSTRACT: Dermal and oral toxicity tests were conducted on brown treesnakes (*Boiga irregularis*) with active ingredients and insecticide formulated products registered with the U.S. Environmental Protection Agency (EPA). Over-the-counter drugs approved by the U.S. Food and Drug Administration (FDA) were evaluated for oral toxicity. Dermal applications of pyrethrin and pyrethroid commercially formulated aerosol insecticides containing the synergists piperonyl butoxide and n-octyl bicyloheptene dicarboximide were toxic to the snakes. The lowest oral gavage dose that resulted in 100% mortality for rotenone, pyrethrins, propoxur, and aspirin was 2.5, 40, 40, and 1,280 mg/kg, respectively; but, when these chemicals were consumed by snakes in bait matrices at doses several times higher than the gavage doses, mortality was greatly reduced. Uncoated tablets of aspirin (150 and 300 mg), ibuprofen (100 and 200 mg), acetaminophen (100 and 200 mg), and commercial over-the-counter tablet formulations of 80 mg and 325 mg acetaminophen were offered to snakes in a dead mouse bait matrix. The mortality with aspirin ranged from 67% to 100%. No mortality was observed with ibuprofen. Acetaminophen resulted in 100% mortality for each of the doses tested. Overall, these dermal and oral toxicity results indicate that some EPA-registered pesticides and drugs approved by FDA may have use as toxicants for brown treesnakes.

KEY WORDS: brown treesnake, *Boiga irregularis,* dermal and oral toxicants, pyrethins, acetaminophen, aspirin, ibuprofen

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INTRODUCTION

The brown treesnake (*Boiga irregularis*) is an invasive species that has caused an unprecedented ecological disaster on the island of Guam (Savidge 1987). Populations of the Marianna fruit bat (*Pteropus mariannus* subsp. *mariannus*) and reptiles were drastically reduced and 9 of 12 native bird species were exterminated (Fritts and Rodda 1998) after the brown treesnake was accidently introduced to Guam after World War II in cargo shipments (Rodda et al. 1992). Brown treesnakes are nocturnal colubrid snakes with estimated population densities of up to 50 to 100 snakes/ha in some areas on Guam (Rodda et al. 1999). In addition to their impacts on native fauna these rear-fanged and mildly venomous snakes pose a health risk to small children (Fritts et al. 1990, 1994), cause power outages by climbing on electrical transmission wires (Fritts et al. 1987), and prey on poultry and other domesticated animals (Fritts and McCoid 1991).

Guam is a hub for commercial and military cargo shipments in the Pacific and there is a serious concern that snakes could be accidently dispersed and breeding populations established in other areas (Fritts et al. 1999; McCoid et al. 1994). In 1993, the Wildlife Services program of the U.S. Department of Agriculture initiated a snake management program in port and cargo facilities to deter the dispersal of snakes from Guam through transportation systems (U.S. Dept. Agric. 1996; U.S. Dept. Int. 1999). Snake management techniques include trapping using live mice as lures (Engeman and Linnell 1998; Engeman et al. 1998a), canine detection (Engeman et al. 1998b; Engeman et al. 1998c), hand capture (Engeman et al. 1999), barriers (Campbell 1999), prey base reduction (e.g., reduction of introduced rodents and birds), and habitat modification (U.S. Dept. Agric. 1996). In addition to these techniques, potential chemical controls have been described for use in an integrated pest management program for brown treesnakes (Campbell et al. 1999). Since 1995 the National Wildlife Research Center has been investigating toxicants (Brooks et al. 1998a), fumigants (Savarie and Bruggers 1999), attractants (Shivik and Clark 1999), and repellents (Clark 1997) for brown treesnakes. The present paper describes some of the dermal and oral toxicants evaluated in our program.

METHODS

Testing was conducted on Guam, and brown treesnakes were captured, maintained, and individually caged as described by Brooks et al. (1998a). Acute dermal toxicity evaluations were conducted with individual toxicants dissolved in 95% ethanol (n=5 snakes per treatment) and with commercial aerosol insecticide formulated products (n=4 snakes per treatment). Dermal doses of 20, 40, and 80 mg/kg were administered at 1 ml per 100 g of body weight with a syringe with a ball-tipped needle. Doses of individual toxicants and aerosol insecticide sprays were applied to the lateral and ventral scales from the neck to the vent of physically restrained snakes and snakes were observed for three days for signs of intoxication or mortality. Aerosol insecticides were sprayed for two seconds and amounts of spray delivered and mg/kg doses were calculated (Brooks et al. 1998b).

Oral toxicants (rotenone, pyrethrins, propoxur, aspirin) were administered by gavage at 1 ml per 100 g of body weight directly into the entrance of the esophagus

using propylene glycol as the carrier with a syringe with a ball-tipped needle. Snakes were observed for three days for signs of intoxication or mortality and in oral bait matrices.

Oral toxicants were also tested in bait matrices. In the first oral bait dosing experiment the toxicants (rotenone, pyrethrins, propoxur, aspirin) were inserted into the bait matrix (processed meat products, quail chicks, young dead mice) and placed in the cages and offered to snakes overnight. Rotenone and propoxur, dissolved in acetone, were added to gelatin capsules in 0.1 to 0.2 ml amounts and the acetone allowed to evaporate before the capsules were inserted into the baits. Pyrethrins, an oily amber-colored liquid, was added to the capsules without evaporation. Commercial aspirin tablets were inserted into baits intact. In the second oral bait dosing experiment, commercial aspirin and acetaminophen tablets, and aspirin, ibuprofen, and acetaminophen tablets formulated by the NWRC Analytical Chemistry Project were inserted into young dead mice. Baits not consumed overnight remained in the cage for a second night and were discarded if not consumed. In the first and second oral bait experiments snakes were observed for three and seven days, respectively, after bait consumption.

RESULTS AND DISCUSSION

Dermal Toxicity

The dermal screening dose of 40 mg/kg, testing active ingredients in aerosol insecticide products registered with the U.S. Environmental Protection Agency, produced brown treesnake mortality in three (pyrethrins, allethrin, resmethrin) of the seven chemicals tested (Table 1). Natural pyrethrins are the active insecticidal constituents of pyrethrum flowers (*Chrysanthemum cinerariaefolium*) and the pyrethroids allethrin and resmethrin are synthetic analogues of the pyrethrins (Davies 1985). Overt signs of intoxication included tremors, lethargy, and loss of righting reflex with the snake lying ventral side up. No snake mortality was observed with: 1) the pyrethroids permethrin, fenvalerate, phenothrin, and tetramethrin; 2) the 80 mg/kg dose of piperonyl butoxide, which is a synergist for pyrethrins and pyrethroids; or 3) the control snakes dosed with ethanol.

Mortality data for eight commercial aerosol insecticides sprayed on snakes is presented in Table 2. All eight insecticides are U.S. EPA General Use registered products and collectively have a broad range of applications from outdoor crawling and flying insects to indoor uses in homes, hospitals, food processing and storage areas, and warehouses. Formulation ingredients and registration numbers for each insecticide are listed in Brooks et al. (1998b). The aerosol spray formulations containing various proportions of pyrethrins, pyrethroids, and the synergists piperonyl butoxide and/or n-octyl bicycloheptene dicarboximide were lethal to 18 of 32 snakes. Signs of intoxication including tremors and disorientation were observed within 1 hr after spraying. Some snakes were moribund in 2 to 3 hr and death usually occurred overnight. Several of the insecticide formulations contained petroleum distillate and its dermal toxicity was tested by applying 2 to 3 ml with a syringe to the bodies of snakes; signs of intoxication were not observed and no snakes died. Also, there was no mortality in untreated control snakes that were handled similarly to the dosed snakes.

Table 1. Dermal toxicity of pyrethrins and pyrethroids to brown treesnakes using ethanol as the carrier (adapted from Brooks et al. 1998a).

	Dose, mg/kg (No. Dead/No. Treated)			
Toxicant	0	20	40	80
Pyrethrins	-	1/5	2/5	-
Allethrin	-	2/5	3/5	-
Resmethrin	-	0/5	1/5	-
Permethrin	-	-	0/5	-
Fenvalerate	-	-	0/5	-
Phenothrin	-	-	0/5	-
Tetramethrin	-	-	0/5	-
Piperonyl butoxide	-	-	-	0/5
Ethanol (Control)	0/15	-	-	-

Table 2. Brown treesnake mortality after spray applications of commercial aerosol insecticides (adapted from Brooks et al. 1998b).

Insecticide Trade Name	Active Ingredients (A.I.)	Estimated Average of A.I. Delivered (mg/kg)	Mortality
Top Crest House and Garden Bug Killer	Resmethrin	38	0/4
	Allethrin	29	
Black Flag House and Garden Insect Killer	Tetramethrin	46	0/4
	Phenothrin	44	
Raid House and Garden Bug Killer	Pyrethrins	50	1/4
	Piperonyl butoxide	210	
Raid Ant and Roach Killer 6	Permethrin	40	2/4
	Pyrethrins	40	
	Piperonyl butoxide	100	
Whitmire X-Clude Encapsulated Natural Pyrethrum PT1600A	Pyrethrins	102	3/4
	Piperonyl butoxide	748	
	n-Octyl bicycloheptene dicarboximide	114	
	Petroleum distillates	412	
Whitmire PT230 Tri-Die	Pyrethrins	193	4/4
	Silica gel	1930	
	Piperonyl butoxide	2573	
Whitmire PT565 Pyrethrin Insect Fogger	Pyrethrins	270	4/4
	Piperonyl butoxide	540	
	n-Octyl bicycloheptene dicarboximide	540	
Whitmire Aero-Cide PT3-6-10 Pyrethrum Insect Fogger	Pyrethrins	320	4/4
	Piperonyl butoxide	640	
	n-Octyl bicycloheptene dicarboximide	1066	
	Refined petroleum oil	2560	
Stoddard Solvent (Control)	Petroleum distillates	2 to 3 ml/ snake	0/4

Dermal toxicity has been reported for pyrethrins and pyrethroid aerosol formulations sprayed on several venomous snakes (Toriba et al. 1999). These investigators recommended that the aerosols be sprayed by animal control personnel directly on snakes, such as the highly venomous habu (*Trimeresurus flavoviridis*), when they are encountered. Fending off the brown treesnake is not as urgent, except if the snake is attacking an infant. In such cases, people usually dispatch the snake by a physical method such as clubbing. As a practical application of dermal toxicants to brown treesnakes, we envision that passive derman aerosol dispensers, if activated by snakes, could be placed in warehouses and cargo staging areas where there is a potential for them to enter into sea and air transportation systems. We are evaluating an infrared (IR) electro/mechanical aerosol dispenser that directs a spray to the body of a snake when the IR beam is tripped by the snake as it crawls into a tube.

Oral Gavage Toxicity

Four chemicals (rotenone, pyrethrins, propoxur, aspirin) resulted in 100% mortality after oral gavage (Table 3). Rotenone, a registered insecticide and piscicide, was the most potent of the four toxicants, killing all snakes at 2.5 mg/kg. The higher doses of rotenone killed snakes within 1 hr; signs of intoxication were a gaping mouth, lethargy, and respiratory distress. Pyrethrins and propoxur resulted in 100% mortality at 40 mg/kg. Muscular tremors and disorientation in 2 to 4 hr were the main signs of intoxication with pyrethrins and the majority of the deaths occurred within 24 to 48 hr. Propoxur, a carbamate in registered insecticides, was fast acting and killed snakes dosed at 40 mg/kg within 1 hr. Loss of righting reflex and lethargy were observed 38 min after dosing, and at death some snakes had heavy secretions from the mouth. Aspirin, a common over-the-counter analgesic drug, killed all snakes at 1,280 mg/kg. No overt signs of intoxication were observed in snakes killed 24 to 48 hr after dosing with aspirin.

Oral Bait Toxicity—Experiment 1

The toxicity of rotenone, pyrethrins, propoxur, and aspirin was greatly reduced when the toxicants were incorporated into bait matrices rather than delivered by gavage (Table 4). None of the 13 snakes that consumed rotenone baits containing 2.5, 5, or 10 mg died or showed signs of intoxication. For average snakes weighing 50 to 100 g, the highest dose of rotenone in bait, 10 mg, is about 40 to 80 times higher than the oral gavage dose of 2.5 mg/kg which killed all the snakes. The 40 mg pyrethrins baits killed 50% of the snakes; this dose is 10 to 20 times greater than the 40 mg/kg oral gavage dose that produced 100% mortality. Two of the four snakes that survived the 40 mg pyrethrins bait were uncoordinated and lethargic but both recovered after 48 hr. Mortality with the 40 mg/kg propoxur oral gavage dose was 100%, but was only 75% for 20 mg baits, which contain 5 to 10n times more chemical than the gavage dose. The 325 mg bait doses of aspirin killed 67% of the snakes; this dose is about 2.5 to 5 times greater than the 1,280 mg/kg gavage dose that resulted in 100% mortality. One possible explanation for the reduced mortality with the high bait doses is that the toxicants were released so slowly from the bait matrices that the snakes could detoxify them before the accumulation of lethal concentrations.

Oral Bait Toxicity—Experiment 2

Aspirin, ibuprofen, and acetaminophen oral bait toxicity results using dead mice as the matrix are shown in Table 5. As in oral bait toxicity experiment I (Table 4), aspirin baits consumed by snakes did not cause 100% mortality for all baits consumed (Table 5). The 325 mg dose in baits represents a 3,250 to 6,500 mg/kg dose, which exceeds the 100% mortality oral gavage dose of 1,280 mg/kg by a factor of 2.5 to 5. No mortality was observed with the ibuprofen baits. Signs of sublethal ibuprofen intoxication within 24 to 36 hr were lethargy, disorientation, and a state of catatonia in which snakes would not strike; but the snakes appeared fully recovered

Table 3. Oral gavage toxicity of toxicants to brown treesnakes using propylene glycol as the carrier (adapted from Brooks et al. 1998a).

	Dose, mg/kg (No. Dead/No. Tested)								
Toxicant	1.25	2.5	5	10	20	40	320	640	1280
Rotenone	1/5	5/5	5/5	5/5	5/5	-	-	-	-
Pyrethrins	-	-	0/5	4/5	3/5	5/5	-	-	-
Propoxur	-	-	0/5	2/5	3/5	5/5	-	-	-
Aspirin	-	-	-	-	-	-	0/5	3/5	5/5
Control	-	-	-	-	-	0/5	-	0/3	-

Table 4. Mortality of brown treesnakes offered toxicants in an oral bait matrix.

Toxicant	Dose (mg)	Bait Matrix	Number of Snakes Offered	Number of Snakes Consumed	% Mortality
Rotenone	2.5	Spam™	8	3	0
Rotenone	5	Spam™	5	1	0
Rotenone	5	Mouse	8	4	0
Rotenone	10	Spam™	5	3	0
Rotenone	10	Quail	5	2	0
Pyrethrins	20	Spam™	5	5	20
Pyrethrins	40	Quail	5	4	50
Propoxur	20	Spam™	5	1	100
Propoxur	20	Quail	10	3	67
Aspirin	325	Spam™	14	8	75
Aspirin	325	Quail	5	1	0

Table 5. Mortality of brown treesnakes offered tableted non-narcotic analgesic drugs in a young dead mouse bait matrix (n=10 snakes per treatment).

Drug	Dose (mg)	No. Baits Consumed	Mortality
Aspirin[1]	150	6	4/6 (67%)
Aspirin[1]	300	7	7/7 (100%)
Aspirin[2]	325	9	6/9 (67%)
Ibuprofen[1]	100	9	0/9 (0%)
Ibuprofen[1]	200	9	0/9 (0%)
Acetaminophen[2]	80	8	8/8 (100%)
Acetaminophen[1]	100	8	8/8 (100%)
Acetaminophen[1]	200	9	9/9 (100%)
Acetaminophen[2]	325	10	10/10 (100%)
Control[1] (inactive table ingredients)	0	10	0/10 (0%)

[1]Tablets formulated at the NWRC.
[2]Tablets purchased over-the-counter.

after four to five days. Acetaminophen resulted in 100% mortality in bait doses ranging from 80 to 325 mg. As oral gavage toxicity studies were not conducted with ibuprofen and acetaminophen, a comparison to the mg/kg dose consumed in the baits cannot be made. The majority of the deaths with acetaminophen occurred in 24 to 48 hr with lethargy being the main sign of intoxication.

SUMMARY

Several pyrethrin aerosol formulation products currently registered with the U.S. EPA for other uses have been identified as dermally toxic for brown treesnakes, but a suitable delivery device for wide-scale application needs to be developed. As these products have a broad range of outdoor and indoor uses, it is highly unlikely that they would have a major impact on the environment or on human health and safety. Acetaminophen, a U.S. FDA approved non-prescription drug in adult and children medications, used in a dead mouse matrix was an effective oral toxicant. It has negligible human health safety concerns. In a non-target assessment using 24 hr video camera surveillance at 231 stations baited with untreated dead mice on Guam, we determined mice being eaten on only two occasions, both monitor lizards (*Varanus indicus*), also an introduced species. These data suggest the baits applied in the field would have minimal impact to other animals.

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