

Risk Assessment of an Acetaminophen Baiting Program for Chemical Control of Brown Tree Snakes on Guam: Evaluation of Baits, Snake Residues, and Potential Primary and Secondary Hazards

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The brown tree snake (*Boiga irregularis*) is a significant ecological, agricultural, and economic pest on Guam. Acetaminophen has recently been identified as a promising snake toxicant. Subsequent experimentation has shown that acetaminophen–mouse baits are readily consumed by and acutely toxic to brown tree snakes. Before implementing an island-wide acetaminophen–mouse baiting program for the reduction of brown tree snake populations, the potential risks to nontarget wildlife must be evaluated. Quantification of nontarget hazards by comparing potential exposure levels to toxicity values suggested a significant level of concern for rodents, cats, pigs, and birds. For these species, subsequent calculations and field and laboratory experiments, which quantified acetaminophen consumption under field conditions, indicated that acetaminophen consumption was minimal. These results indicate that the advantages of using acetaminophen to reduce brown tree snake populations on Guam outweigh the minimal risks to nontarget feral and wildlife species.

Introduction

The brown tree snake (*Boiga irregularis*) is a nocturnal, arboreal, rear-fanged, mildly venomous, colubrid snake that can reach lengths of up to 2.3 m and weigh as much as 2 kg (1, 2). Originally, the species' range included the northern and eastern coasts of Australia, Papua New Guinea, and nearby islands (3). It is believed that sometime in the 1950s brown snakes were inadvertently transported from New Guinea to Guam, where they proliferated (4, 5). By the mid-1960s, marked decreases in Guam's bird life were observed. By the mid-1980s, snake densities were estimated at 50–100/ha (13 000–26 000/mi²), higher densities than those recorded for any other snake (4, 6, 7).

Brown tree snakes are dietary generalists. They have been observed to eat chicken bones, cooked spare ribs, lizards, birds, rodents, domestic fowl hatchlings, puppies, piglets,

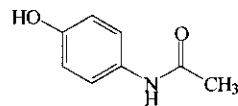


FIGURE 1. Acetaminophen.

rabbits (in hutches), and pet birds (in cages inside homes) (2, 8, 9). Human infants have also been attacked, resulting in very serious bites (10). Snake predation has resulted in the decline and extirpation of Guam's avifauna and herpetofauna (1, 11). Only eight Mariana crows (*Corvus kubaryi*) remain on Guam, with an additional 300–600 remaining on the nearby island of Rota (12, 13). In addition to the ecological and agricultural damage, snakes crawl along power lines in search of prey. This activity frequently results in short circuits leading to extensive damage to power transmission equipment, subsequent power blackouts to human population centers, and millions of dollars in economic losses (14).

The large military presence on Guam and shipment of associated cargo coupled with the high snake densities increase the likelihood of dispersal of the snake to other locations where the whole damage scenario might be repeated. Individual brown tree snakes have been observed on other islands such as Kwajalein, Wake, Diego Garcia, Saipan, Tinian, and Hawaii (15, 4). One snake was found in a cargo container in Corpus Christi, TX, that had been shipped from Guam some 7 months earlier (16).

The United States Department of Agriculture Wildlife Services personnel on Guam utilize a variety of measures such as trapping and snake detector dogs to prevent accidental snake relocations (17), but the only long-term solution is the significant reduction or eradication of the brown tree snake population on Guam. As part of a snake control research program funded by the U.S. Department of Defense's Legacy Program, scientists from the National Wildlife Research Center (NWRC) have evaluated traps, lures, repellents, and chemical toxicants. While a number of potential toxicants were evaluated for effectiveness against brown tree snakes, acetaminophen (*N*-(4-hydroxyphenyl)-acetamide) (Figure 1) appears to be the most promising (18). Additionally, in an evaluation of multiple bait matrices, it was shown that dead mice were an excellent attractant for brown tree snakes (19, 20).

Acetaminophen-fortified dead neonatal mice have been shown to be an effective oral toxicant to brown tree snakes under both laboratory and field conditions (21). Studies have also been conducted to determine the potential nontarget hazards associated with an acetaminophen baiting program. These include estimating the potential exposure of nontarget species (Table 1) to the acetaminophen baits by determining the stability of the baits under field conditions, estimating the potential exposure to nontarget scavengers feeding on snake carcasses, and determining the magnitude and stability of acetaminophen residues in successfully baited brown tree snakes under field conditions. These data were then used to make assessments regarding potential primary and secondary hazards to the endangered Mariana crow and other potential scavengers on Guam. As an endangered species and a scavenger that might consume acetaminophen–mouse baits and/or snake carcasses resulting from chemical toxicant control operations, the Mariana crow plays a significant role in secondary hazard assessments of the use of such toxicants. Should brown tree snakes inadvertently be introduced to other islands, these residue data and the related hazard assessments will also assist researchers in estimating potential nontarget hazards to the indigenous wildlife.

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TABLE 1. Primary (Exposure to Treated Mouse Baits) and Secondary (Exposure to Snakes Killed by Acetaminophen) Hazard Estimates to Nontarget Species

species	acute toxicity estimate ^a (mg/kg)	food consumption rate (g of food/g of body wt)	primary hazard		secondary hazard	
			acetaminophen dose ^b (μg/g)	hazard quotients ^c	acetaminophen dose ^d (μg/g)	hazard quotients ^c
Mammals						
house mouse (<i>Mus musculus</i>)	LD ₅₀ = 338 ^e	0.15 ^m	2400	7.12	132.2	0.39
Norway rat (<i>Rattus norvegicus</i>)	LD ₅₀ = 1944 ^f	0.10 ^m	1600	0.80	88.1	0.05
guinea pig (<i>Cavia cobaya</i>)	LD ₅₀ = 2620 ^g	0.10 ⁿ	1600	0.61	88.1	0.03
domestic dog (<i>Canis familiaris</i>)	LD ₅₀ > 2000 ^h	0.06 ^o	960	<0.48	52.9	<0.03
domestic cat (<i>Felis catus</i>)	LD ₅₀ = 361 ⁱ	0.07 ⁿ	1005	2.78	61.7	0.17
pig (<i>Sus scrofa</i>)	LD ₅₀ > 1000 ^j	0.08 ^p	800	<0.8	44.1	<0.04
Birds						
northern bobwhite (<i>Colinus virginianus</i>)	LD ₅₀ > 2250 ^k	0.08 ^q	1280	<0.57	70.5	<0.03
fish crow (<i>Corvus ossifragus</i>)	LD ₅₀ > 560 ^l	0.08 ^r	1280	<2.29	70.5	<0.10

^a LD₅₀, lethal dose, 50% kill. ^b Based on the nominal acetaminophen concentration found in mouse baits, 80 mg of acetaminophen/5 g of mouse (16 000 μg/g). ^c Hazard quotient = acetaminophen dose/acute toxicity estimate. ^d Based on the calculated theoretical highest acetaminophen concentration in a snake killed by acetaminophen, 881 μg/g. ^e Ref 43. ^f Ref 44. ^g Ref 45. ^h Ref 32. ⁱ Ref 32. ^j Ref 40. ^k Ref 35. ^l Ref 34. ^m Ref 46. ⁿ Ref 29. ^o Ref 47. ^p Refs 41 and 48. ^q Ref 31. ^r Ref 41.

Materials and Methods

Chemicals. USP acetaminophen was obtained from Aerchem (Bloomington, IN). Cross-linked poly(vinylpyrrolidone) and carboxymethyl cellulose (sodium salt) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Calcium phosphate dibasic and stearic acids were obtained from Spectrum Chemical Co. (Gardena, CA). Avicel was obtained from FMC (Princeton, NJ), and magnesium stearate was obtained from Witco Oleo Surfactants (Greenwich, CT). Potassium phosphate monobasic was obtained from Mallinckrodt Chemical (Paris, KY). HPLC-grade ethanol and methanol were obtained from EM Science (Gibbstown, NJ). HPLC-grade acetone, ethanol, methanol, and water were obtained from Fisher Scientific (Fair Lawn, NJ).

Production of Acetaminophen Tablets. Tablets containing 40 mg of acetaminophen were made by preparing a homogeneous dry mix of 82.65% acetaminophen, 4.55% poly(vinylpyrrolidone), 3.97% carboxymethyl cellulose, 5.2% avicel, and 2.72% calcium phosphate. The dry mix was combined with approximately 50 wt % of water to form a homogeneous paste. The paste was pressed through a no. 8 sieve, and the resulting strands were cut in to lengths of approximately 2 in. The strands were dried to approximately 3% moisture at 60 °C. The dried strands were then sieved through a no. 20 sieve and mixed with magnesium stearate and stearic acid at 0.3 and 0.61 wt %, respectively. The mixture was formed into tablets using a Stokes/Pensault (Warminster, PA) model E tablet press. Tablets were 5 mm (diameter) × 2.5 mm (height) and weighed approximately 55 mg.

Isolation of Acetaminophen from Tablets. An acetaminophen tablet was weighed in a tared 10-mL tube. Following the addition of 2 mL of water, the tablet was permitted to dissociate. Methanol (8 mL) was added to the tube. The tube was vortex-mixed (30 s), sonicated (5 min), mechanically shaken (10 min), and centrifuged at 1700g for 10 min. A 0.250-mL aliquot of the supernatant was transferred to a 10-mL volumetric flask. Methanol:water (80:20) was added to volume. The acetaminophen content of the tablets was determined by HPLC analysis of 5-μL aliquots of this solution. The target quantity of the acetaminophen in the prepared tablets was 40 mg; the method was validated by extracting blank tablets fortified with 30 and 48 mg of acetaminophen (22).

Storage Stability and Field Stability of Acetaminophen-Mouse Baits. In a brown tree snake operational baiting program, it is anticipated that the acetaminophen-mouse baits will be prepared in large batches and stored frozen for up to several weeks prior to use. To evaluate the stability of

acetaminophen in the frozen mouse, 15 acetaminophen-mouse baits were prepared. The acetaminophen concentration was determined immediately in three baits. The remainder of the baits were stored at -13 °C. The acetaminophen concentrations were determined in triplicate mice removed at 1, 2, 3, and 7 weeks of frozen storage.

It was also imperative to evaluate the stability of the acetaminophen-mouse baits under field conditions as the baits may be exposed to the environment for several days prior to being consumed by a snake. To address this scenario, we evaluated the stability of the baits under simulated field conditions. Twelve acetaminophen-mouse baits were prepared and placed in an environmental chamber. Replicates were also conducted with previously frozen baits. To simulate environmental conditions on Guam, the chamber temperature was cycled between 30 °C/60% relative humidity for 14 h and 25 °C/50% relative humidity for 10 h. The acetaminophen content of the mouse baits were determined at 0, 1, 2, and 3 days.

Analysis of Acetaminophen in Mouse Baits. Each mouse bait was placed in a 50-mL screw-top test tube containing 15 mL of ethanol:water (1:1). The test tube contents were homogenized for approximately 3 min using a Brinkman (Westbury, NY) Polytron and 10 × 185 mm generator probe. Following homogenization, 10 mL of the ethanol:water solution was added to the tube. The tube was capped, vortex-mixed for several seconds, sonicated for 10 min, high-speed-mixed on a mechanical shaker, and sonicated for two additional 10-min periods interspersed with vortex mixing. The sample was centrifuged for 10 min at 1700g. The supernatant was decanted into a 50-mL volumetric flask. The mouse-acetaminophen homogenate pellet was extracted two more times with 10-mL aliquots of the ethanol:water solution. Each extraction was followed by vortex-mixing, sonication, and centrifugation. The extracts were added to the volumetric flask, which was then brought to volume with the ethanol:water solution. The mouse bait extract was volumetrically diluted 0.250-10 mL with methanol:water (60:40). Acetaminophen in the mouse baits was quantified by HPLC analysis of 2-μL aliquots of the diluted mouse bait extract. Acetaminophen concentrations between treatment groups were compared using two-tailed *t* tests (23).

In-Life. In June 2000, a study was conducted on Guam to determine the magnitude and stability of acetaminophen residues in baited brown tree snakes. Forty-five brown tree snakes were obtained from USDA Wildlife Services operational trapping activities on Guam. Thirty brown tree snakes

were assigned to the treatment group and offered dead neonatal mice (Falcon Labs, Van Nuys, CA) containing two tablets, each of which contained 40 mg of acetaminophen. Fifteen snakes were assigned to the control group and offered dead neonatal mice containing two tablets without acetaminophen.

Six treated and three control snakes of either sex were randomly assigned by bodyweight to the 0, 1, 2, 3, or 4 day post-mortem environmental exposure groups. Snakes were individually caged and offered one dead neonatal mouse bait overnight as the only food source. If a snake did not consume the bait during the first night, the bait remained in the cage for a second night. Bait not consumed after the second night was removed and replaced with a fresh bait that was offered for a maximum of two additional nights. Snakes that did not consume baits after 4 nights were euthanized.

Snakes were checked at approximately noon on each day. Dead snakes were removed, weighed, sexed, and measured (snout-vent length). Post-mortem day 0, 1, 2, 3, and 4 snakes were placed on uniquely numbered aluminum foil boats of known weight and transported to the field where they were placed on a 8 in. high \times 8 in. diameter 1/4 in. hardware mesh support. The snakes on the mesh support were then placed under a 1/4 in. hardware mesh wood-framed cage, the top of which was covered with a tarpaulin to prevent scavengers from disturbing the snake carcasses and to keep rain off the carcasses. Using this post-mortem procedure in the field, the snakes were exposed to the ambient microflora, insects, temperature, and relative humidity. After the prescribed post-mortem number of days in the field, snakes were returned to the lab, weighed, wrapped in aluminum foil, and placed in uniquely numbered plastic bags. Snakes were stored at -20°C on Guam until transported with ice packs in an insulated container to the NWRRC. Upon receipt, snakes were stored at -16°C until analyzed.

Analysis of Acetaminophen in Brown Tree Snake Carcasses. Prior to quantifying the acetaminophen content in brown tree snake carcasses, the mass of each snake carcass was determined. Whole body brown tree snakes were initially homogenized by manually pulverizing 1–2-in. pieces of frozen carcasses in liquid nitrogen (24). Aliquots (1.00 g) of the homogenate were placed in a 50-mL glass screw-top test tube, combined with 10 mL of acetone, briefly vortex-mixed, and mechanically shaken for 10 min. The tubes were then sonicated for 10 min and centrifuged (1700g) for 5 min. Aliquots (1.0 mL) of the extract were transferred to a 15-mL glass centrifuge tube and evaporated to dryness under a gentle stream of nitrogen at 60–65 $^{\circ}\text{C}$. The residue was reconstituted in 2 mL of 50 mM monobasic potassium phosphate in 15:85 methanol:water (pH 3.2). This solution was vortex-mixed, sonicated, and filtered. The acetaminophen content was determined by HPLC analysis of 25- μL aliquots of this solution (25). Acetaminophen concentrations were compared using analysis of variance, *t* tests, and/or linear regression as appropriate (23, 26).

High-Performance Liquid Chromatography (HPLC). HPLC analyses were performed on a Hewlett-Packard (Palo Alto CA) 1090M HPLC equipped with an ultraviolet diode array detector (250 nm). For the analysis of the tablet extracts, a 250 mm \times 4.6 mm (i.d.) Alltech Associates (Deerfield, IL) C18 Econosil column was used with a methanol:water (75:25) mobile phase at a flow rate of 1.0 mL/min. The column was at room temperature, and the chromatographic run time was 8 min. Identical conditions were used for the analysis of the diluted mouse bait extracts except that the mobile phase was methanol:water (60:40). This necessitated an increased chromatographic run time of 10 min. For analysis of snake carcasses extracts, the HPLC was equipped with a 250 mm \times 4.6 mm (i.d.) Phenomenix (Torrance, CA) Prodigy

C₁₈ column. An isocratic mobile phase of 50 mM monobasic potassium phosphate in 15:85 methanol water (pH 3.2) was used at a flow rate of 1.0 mL/min. The column temperature was maintained at 25 $^{\circ}\text{C}$. Chromatographic run time was 20 min.

Results and Discussion

Quantification of Acetaminophen. Linearity of chromatographic response was assessed for each of the chromatography conditions required for this study. In each case, a range of acetaminophen standard solutions at concentrations including the expected acetaminophen concentrations in quality control and field samples was analyzed. In all cases, linear regression analysis of concentration versus response yielded an r^2 greater than 0.99. Linear regression analysis of the log:log plot of these data yielded a slope that was not significantly different than 1 ($P > 0.05$). These data indicate that the chromatographic responses for acetaminophen were linear and proportional, which justified the use of single-point external standard for quantification of acetaminophen in the sample extracts.

A method limit of detection (MLOD) was calculated for each matrix as the acetaminophen concentration required to produce a chromatographic response that was equivalent to three times the baseline noise at the retention time of acetaminophen. These MLODs were calculated using the response factors for acetaminophen-fortified control matrices. The acetaminophen MLODs were 0.72 $\mu\text{g/g}$ for brown tree snake tissue, 3.7 $\mu\text{g/g}$ for the mouse baits, and 2.5 mg/tablet for tablets. These limits of detection indicate that the analytical methodology was sufficiently sensitive to monitor the acetaminophen concentrations in the tablets, baits, and homogenized snake tissue.

Tablets. The analytical method for the quantification of acetaminophen in tablets yielded mean recoveries greater than 95% and coefficient of variation (CV) less than 3.7% for the blank tablets fortified with acetaminophen at 30 and 50 mg per tablet. Analyses of the acetaminophen tablets used for this study indicated a mean acetaminophen concentration of 42.8 mg per tablet with a CV of 4.2%. As this value is not significantly different than the target value of 40 mg per tablet (two-tailed *t* test, $p = 0.003$), it was assumed that all snakes were dosed at the nominal value of 40 mg per tablet.

Mouse Baits. For the validation of the analytical method for the quantification of acetaminophen in mouse baits, the target concentration of 80 mg of acetaminophen per bait was bracketed by analyzing mice fortified with approximately 40, 80, and 120 mg of acetaminophen per mouse. Acetaminophen recoveries ranged from 89.5% to 91.9% with an overall mean recovery of 90.7%. The CV for all fortification levels was less than 2.7%. These data suggest that the analytical method was well suited for the quantification of acetaminophen in mouse baits.

Snakes. To validate the analytical method for the quantification of acetaminophen in brown tree snakes, 1-g replicates of control brown tree snake tissue were fortified with acetaminophen at 19.1 or 2400 $\mu\text{g/g}$. Mean recoveries were 92.5% (CV = 2.1%) at the lower level and 93.4% (CV = 1.3%) at the higher level. Analysis of nonfortified control tissue indicated that there were no chromatographic interferences at the retention time of acetaminophen.

Storage Stability of Acetaminophen—Mouse Baits. Acetaminophen—mouse baits were removed from frozen storage (-13°C) and analyzed for acetaminophen following 0, 1, 2, 3, and 7 weeks of storage (Table 2). Linear regression analysis (23) of acetaminophen concentration versus weeks of storage resulted in a slope that was not significantly different than zero ($P = 0.216$). Additionally, comparison of

TABLE 2. Storage and Field Stability of Acetaminophen—Mouse Baits

time	% recovery	
	mean	SD
storage at -13 °C		
0 weeks	89.1	2.3
1 week	89.8	2.0
3 weeks	82.6	1.5
7 weeks	86.1	2.7
field conditions: frozen		
0 days		
1 day	98.3	2.8
2 days	93.7	3.0
3 days	91.1	1.4
field conditions: fresh		
0 days		
1 day	96.5	2.6
2 days	91.5	1.1
3 days	90.5	1.1

mean acetaminophen concentrations by *t* test indicated that the acetaminophen concentration at week 0 was not different than at week 7 ($P = 0.178$). These data suggest that the acetaminophen content of frozen baits is stable for up to 7 weeks of frozen storage.

Field Stability of Acetaminophen—Mouse Baits. To determine the stability of acetaminophen—mouse baits under field conditions, baits were removed from the environmental chamber and analyzed for acetaminophen at days 0, 1, 2, and 3. At each sampling time, three baits that had been frozen for 1 week prior to the beginning of the experiment and three baits that were prepared immediately prior to the beginning of the experiment were removed from the environmental chamber and analyzed for acetaminophen. Statistical analysis (*t* tests) indicated that there was no difference between the field stability of acetaminophen in freshly prepared and previously frozen baits ($P = 0.274-0.570$). Linear regression analysis of acetaminophen concentration in the pooled frozen and fresh mouse baits versus days in the environmental chamber resulted in a slope of -2.43 that was significantly different than zero ($P < 0.001$) (Table 2). This indicated that the acetaminophen content in the mouse baits decreased by about 2.4% per day under simulated Guam environmental conditions. Since acute toxicity tests indicated that acetaminophen doses of 40 mg were lethal to 100% of dosed snakes (27), a 7–8% decrease of the 80 mg of acetaminophen dose in mouse baits (over 3 days) should not impact efficacy. In general, the bait matrix was totally decomposed by day 4. These storage and field stability tests indicated that 80 mg of acetaminophen—mouse baits can be prepared in advance, frozen for nearly 2 months, and will remain viable under field conditions for 3 days.

Brown Tree Snake Consumption of Mouse Baits. In the study to determine the magnitude and stability of acetaminophen residues in successfully baited brown tree snakes, approximately one-third of the snakes that consumed the acetaminophen—mouse baits on Guam regurgitated the baits shortly after consumption. The mean total acetaminophen body burden for snakes that regurgitated the bait was less than 0.5% of the administered dose as compared to 20.9% of the administered dose in the snakes that retained the bait. While the acetaminophen residues were significantly lower in the snakes that regurgitated the bait (*t* test, $P = 4.5 \times 10^{-6}$), every snake that consumed a bait died within 3 days post-consumption. Additionally, the time to death following bait ingestion had no significant effect on acetaminophen residues in the snake homogenates (ANOVA, $P = 0.83$).

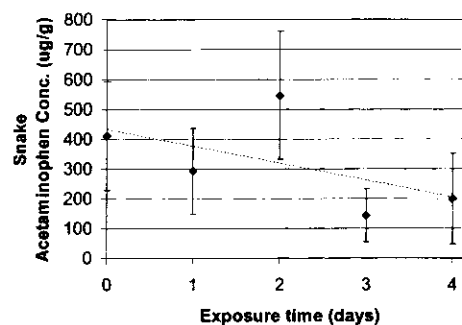


FIGURE 2. Acetaminophen residues in brown tree snakes vs days of exposure to environmental conditions on Guam. Error bars = ± 1 standard deviation.

The residue data from the analysis of acetaminophen-poisoned snakes that had been subsequently exposed to environmental conditions on Guam indicated that acetaminophen residues decreased with increased time of exposure (Figure 2). Linear regression of mean acetaminophen body concentration versus days of exposure generated a r^2 of 0.4 and a slope of -57.2 ($P = 0.33$). A visual inspection of the linear regression plot illustrates that, while the acetaminophen concentration in poisoned snakes was quite variable, acetaminophen concentrations decreased by about 9.5% per day.

Nontarget Hazards. Nontarget wildlife species could be exposed to acetaminophen in conjunction with a brown tree snake baiting program on Guam. As depicted in Figure 3, primary exposure to acetaminophen could result from the direct consumption of the treated mouse baits. Secondary exposure could result from the consumption of dead or dying snakes poisoned by acetaminophen (28). Species potentially consuming either treated mouse baits or scavenging acetaminophen killed snake carcasses include feral dogs and cats, wild pigs (*Sus scrofa*), small rodents, monitor lizards (*Varanus indicus*), crabs, and the endangered Mariana crow and Guam rail (*Gallirallus owstoni*). With the exception of the crow, rail, and crabs, all these species were either intentionally or unintentionally introduced on Guam, have significant negative impacts on the native ecosystem, and are considered pests (5).

The calculation of acute hazard quotients is a screening approach used by the U.S. EPA for evaluating theoretical or worst-case potential hazards to nontarget species (29). An acute hazard quotient is calculated by dividing the expected dietary dose by some measure of acute toxicity, usually the median lethal dose (LC_{50} or LD_{50}) for potentially exposed species. For all species, hazard quotients greater than 0.5 suggest that there may be significant risk to nontarget species associated with the proposed use of a chemical. For endangered species, a hazard quotient greater than 0.1 suggests that the nontarget risks associated with the use of the chemical may be unacceptable to regulatory agencies such as the U.S. EPA (30). In these cases, a more detailed risk assessment is warranted to better elucidate adverse risk to species of concern. The U.S. EPA registration of chemicals with hazard quotients above these levels may be accompanied by use restrictions to minimize exposure to nontarget species (31).

Acetaminophen is a commonly used human analgesic. Consequently, there is a wide range of research available that examines the risk to humans. However, because acetaminophen has never been used as a pesticide, there is a paucity of acute toxicity data (LC_{50} or LD_{50}), for wild species. Because of the lack of acute toxicity data, a variety of toxicity estimates were used in this theoretical screening hazard assessment. For example, accidental poisoning cases were used to determine lethal acetaminophen doses to domestic

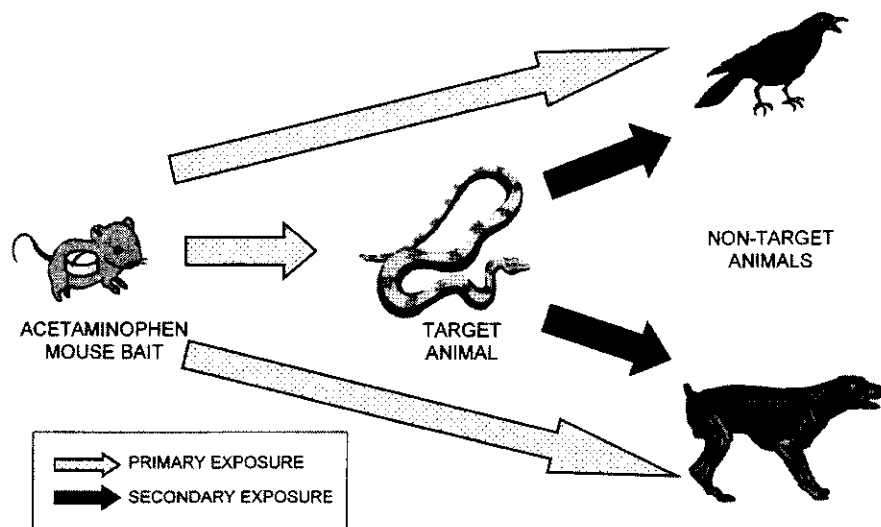


FIGURE 3. Potential routes of primary and secondary exposure for target and nontarget species.

cats. In two separate incidents, cats were administered acetaminophen at 210 and 232 mg/kg. Both cats survived. In a third incident, one of two cats dosed with acetaminophen at 361 mg/kg died. On the basis of these data, we estimated the LD₅₀ for acetaminophen to cats to be 361 mg/kg. However, as the cats in these case studies were administered various therapeutic agents such as Ringer's solution and high caloric diets to combat dehydration and anorexia, this estimated LD₅₀ may underestimate the toxicity of acetaminophen to cats in the wild (32). Likewise a published LD₅₀ was not available for the domestic dog or the fish crow. It was reported that the lowest dose administered to dogs and fish crows that caused death (but less than 50% mortality) was 2000 and 729 mg/kg, respectively (33, 34); therefore, the LD₅₀ for these species are greater than those values. The LD₅₀ value reported for the northern bobwhite was obtained in a definitive acute toxicity study (35). In this study, five dose levels were tested, the highest being 2250 mg/kg. None of the 50 birds receiving doses of acetaminophen died or exhibited any signs of illness during the study. Consequently, the LD₅₀ for the northern bobwhite quail is greater than 2250 mg/kg.

Primary Hazards to Nontarget Wildlife. A screen for potential primary nontarget hazards was conducted using the theoretical worst-case exposure estimates (Table 1). In calculating primary hazard quotients, it was assumed that the daily diet of the nontarget species consisted entirely of bait containing 80 mg of acetaminophen/5 g of mouse. This is equivalent to a diet containing acetaminophen at a concentration of 16 000 µg/g of food. Primary hazard quotients for all species, except the dog, exceed the U.S. EPA's acute hazard threshold of 0.5 (Table 1), suggesting that a more detailed risk assessment is warranted. Such a risk assessment must consider the biology of potentially exposed animals. The most basic of these considerations is the dietary preferences of a species. For example, a reasonable nontarget risk assessment must consider whether a species will scavenge the bait, consume the acetaminophen tablets inserted into the mouse, and/or be able to locate and consume enough baits in 1 day to equal a toxic dose of acetaminophen.

In a pen study of wild caught fish crows, Avery et al. (34) offered mice treated with two 40-mg acetaminophen tablets to crows. The crows readily consumed the mouse tissue but avoided ingesting the tablets. When the crows encountered a tablet, they picked the tablet from the carcass and either set the tablet aside or dropped it from the perch. In a followup study, acetaminophen tablets were force-fed to five crows. No mortality resulted from force-feeding 80 mg of acetaminophen (two acetaminophen tablets), which is the intended bait concentration (34).

When five additional crows were force-fed four acetaminophen tablets (160 mg of acetaminophen), all crows regurgitated the tablets. However, one crow died 3 h after regurgitation. Assuming that fish crows and Mariana crows are equally sensitive to the effects of acetaminophen, it appears that Mariana crows consuming two acetaminophen-treated mouse baits (160 mg of acetaminophen) could theoretically be exposed to a lethal dose of acetaminophen. However, this risk is significantly reduced if the two species exhibit similar foraging behavior and reject the tablets.

Small rodents, rats and mice, could also be exposed to treated baits. Laboratory data show that the house mouse is nearly six times more sensitive to acetaminophen than the Norway rat. Primary acute hazard quotients for these species indicate significant potential risk to the mouse. In fact, if the acetaminophen is evenly distributed inside the bait, a mouse would only need to eat approximately 0.42 g of the mouse bait (1/7th of its daily food requirement) to ingest a dose equivalent to the LD₅₀. Risk to the mouse is dependent upon whether it will consume a dead mouse, and if it does, what portion of the bait it consumes.

In a study conducted on Guam, Savarie (27) evaluated the potential hazard that acetaminophen baiting presents to crabs. Dead mice treated with either two control tablets or two 40-mg acetaminophen tablets (80 mg of acetaminophen) were offered to coconut crabs (*Birgus latro*) and hermit crabs (*Coenobita brevimanus*). Results of this test showed that all crabs of both species survived the exposure. In both tests, crabs consumed the mouse tissue but generally avoided eating the control or acetaminophen tablets.

Guam rails were once abundant but are now confined to a few individuals in a forested snake-excluded area (36). While the sensitivity of rails to acetaminophen is unknown, it is also unknown if wild rails would feed on acetaminophen-mouse baits or snake tissue as their diet consists of vegetation, insects, and worms (37, 38). In a study conducted on Guam, Campbell (39) presented untreated mice to two test groups of captive Guam rails. One test group had been reared on dead mice; the other had not. Campbell observed that the rails that were unfamiliar with mice as a food source, chose not to eat the mice, while the zoo raised, mouse fed rails readily consumed the dead mice. These findings suggest that is unlikely that wild Guam rail populations would initially ingest acetaminophen-mouse baits. However, it is possible that such behavior would change if rails continued to be exposed to acetaminophen-mouse baits.

The wild pig could also be exposed to acetaminophen baits on Guam. No acute oral toxicity data are available for pigs; however, one study reports an LD₅₀ (lowest lethal dose) of 1000 mg/kg for administration via intravenous injection (40). Because intravenous injection eliminates impediments to adsorption caused by the digestive tract, significantly lower concentrations of a compound are generally required to induce toxic effects. However, if one conservatively assumes similar sensitivity between oral and intravenous administration, a theoretical worst-case hazard quotient can be calculated. At a daily food consumption rate of approximately 0.05 g of food/g of body weight (41), a 50-kg pig would consume 2.5 kg of food a day. If a pig's diet consisted entirely of baits (80 mg of acetaminophen/5 g of bait), it would consume a total of 40 000 mg of acetaminophen. A hazard quotient calculated from this scenario is 0.8. It is easily imagined that a pig would eat the entire mouse and not remove the acetaminophen tablet. However, to obtain this dose, a 50-kg pig would have to locate and eat 500 mice in 1 day. For the current baiting directions, baits must be placed no closer than 20 m apart. At this maximum density, the pig would have to find and eat every bait in a 20-ha area to ingest this dose.

As the hazard quotients in Table 1 indicate, baiting snakes with acetaminophen-treated mice presents a significant hazard to cats. However, for a primary hazard to be a significant risk, the nontarget species must consume the acetaminophen in the baits. During the first half of 2001, NWRC scientists used video cameras to monitor consumption of control mouse baits by nontarget wildlife on Guam (42). During 520 h of video monitoring, control mice baits were observed to be removed by brown tree snakes, hermit crabs, and coconut crabs. It is important to note that even though the monitoring sites were within the range of the Mariana crow, mice, dogs, and cats, none of these nontarget species were observed to take the baits. Additionally, as previously noted, crabs and crows avoided the acetaminophen tablets during the consumption of mouse baits. These observations strongly suggest that the primary risks to nontarget wildlife are significantly less than the theoretical worst-case hazard estimates.

Secondary Hazards to Nontarget Wildlife. Another route of exposure is through the consumption of dead or dying snakes. The degree of risk would hinge upon the accessibility of snake carcasses to scavengers and the acetaminophen concentration in snake carcasses. The highest acetaminophen residue detected in snakes in this study was 734.8 µg/g. This residue was detected in a snake that had been exposed to the environment for 2 days. On the basis of the calculated acetaminophen residue decrease rate of 9.5% per day, the acetaminophen concentration for this snake on day 0 would have been approximately 881 µg/g. By assuming that scavenging wildlife would consume a daily diet containing acetaminophen concentration of 881 µg/g, one can determine worst-case nontarget hazards for wildlife potentially consuming acetaminophen-poisoned snake carcasses. As indicated in Table 1, acute hazard quotients for secondary exposure are less than 0.5 for all species, suggesting an acceptable level of risk for nonendangered species. The calculated secondary exposure hazard quotient for the fish crow is less than 0.1, the hazard quotient limit of concern for endangered species. If the fish crow can be used as a surrogate for the Mariana crow, this hazard quotient suggests an acceptable level of risk for the endangered Mariana crow.

NWRC scientists also used video cameras to monitor consumption of brown tree snake carcasses by nontarget wildlife on Guam (42). During 858 h of video monitoring, snake carcasses were observed to be fed upon by hermit crabs and removed from the field of view by coconut crabs. Even though the video monitoring occurred in areas con-

taining the Mariana crow, mice, dogs, and cats, none of these species were observed consuming the carcasses. Secondary toxicity tests conducted by Savarie (27) also indicated little risk to crabs. In these tests, coconut crabs were given ground snakes that had died following consumption of mice containing 160 mg of acetaminophen, twice the dose used in the field. Crabs ate the ground snake, but no signs of toxicosis or mortality were observed. These observations suggest that the secondary risks to nontarget animals are significantly less than the theoretical worst-case hazard estimates and, like primary risks, are likely acceptable.

The use of acetaminophen-treated mice as baits to control brown tree snake populations on Guam carries some degree of theoretical risk to nontarget species. Therefore baiting strategies that minimize potential primary nontarget risks may need to be considered. Such potential nontarget hazard considerations should not be restricted to Guam as registered brown tree snake toxicants may be needed to control future snake populations at other locations. Reducing introduced snake populations from island ecosystems will almost certainly have positive impacts on native ecosystems. Like snakes, most mammal species found on Guam are relatively recent introductions to the island and, in many circumstances, are considered pests. Certainly, pigs, rats, and feral cats have had a significant negative impact on native flora and fauna. As the goals of brown tree snake control include the re-introduction of extirpated native wildlife, expanding the range of native species, and/or slowing the spread of brown tree snakes to other island ecosystems, the significant benefits of an acetaminophen baiting program appear to outweigh the negligible risks to feral or other nontarget wildlife on Guam.

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