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**Ecotoxicology**

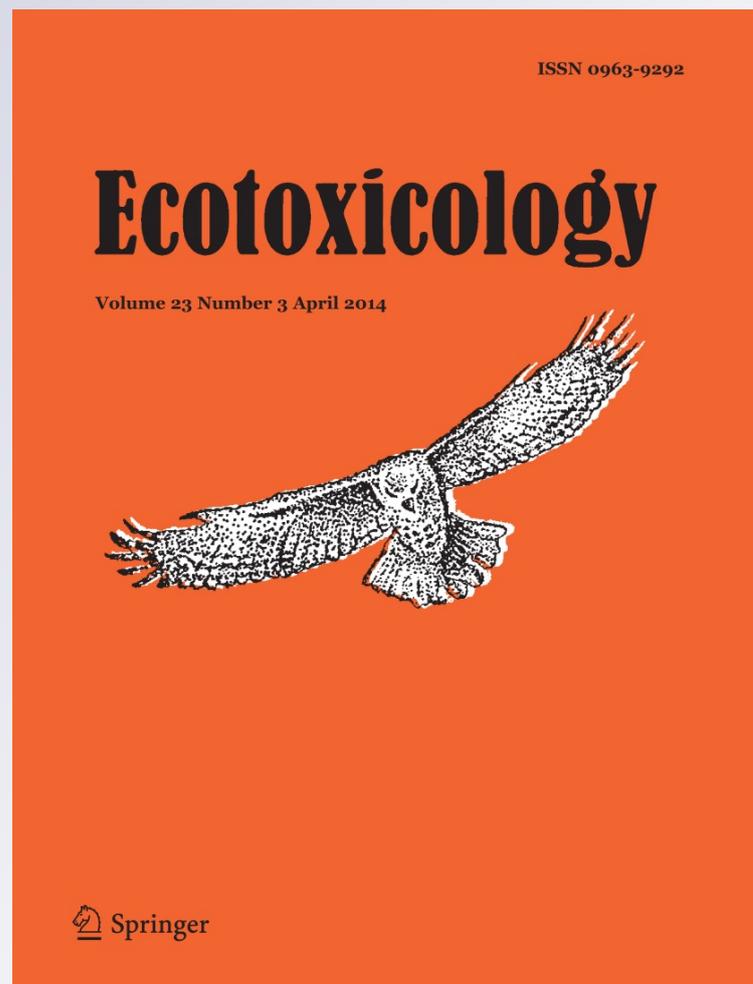
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# Potential citric acid exposure and toxicity to Hawaiian hoary bats (*Lasiurus cinereus semotus*) associated with *Eleutherodactylus* frog control

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Susan M. Jojola · Hans Sin

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**Abstract** We examined potential exposure of Hawaiian hoary bats (*Lasiurus cinereus semotus*) to citric acid, a minimum risk pesticide registered for control of invasive *Eleutherodactylus* frog populations. Hoary bats are nocturnal insectivores that roost solitarily in foliage, federally listed as endangered, and are endemic to Hawaii. Oral ingestion during grooming of contaminated fur appears to be the principal route by which these bats might be exposed

to citric acid. We made assessments of oral toxicity, citric acid consumption, retention of material on fur, and grooming using big brown bats (*Eptesicus fuscus*) as a surrogate species. We evaluated both ground application and aerial application of 16 % solutions of citric acid during frog control operations. Absorbent bat effigies exposed to ground and aerial operational spray applications retained means of 1.54 and 0.02 g, respectively, of dry citric acid, although retention by the effigies was much higher than bat carcasses drenched in citric acid solutions. A high dose delivered orally (2,811 mg/kg) was toxic to the big brown bats and emesis occurred in 1 bat dosed as low as the 759 mg/kg level. No effect was observed with the lower doses examined ( $\leq 542$  mg/kg). Bats sprayed with 5 ml of 16 % (w/w) citric acid solution showed no evidence of intoxication. In field situations, it is unlikely that bats would be sprayed directly or ingest much citric acid retained by fur. Based on our observations, we believe Hawaiian hoary bats to be at very low risk from harmful exposure to a toxic dose of citric acid during frog control operations.

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**Keywords** Hoary bat · *Lasiurus cinereus semotus* · Coqui frog · *Eleutherodactylus coqui* · Control · Risk · Citric acid · Hawaii

## Abbreviations

|           |   |
|-----------|---|
| APHIS     | Animal and Plant Health Inspection Service        |
| FIFRA     | Federal Insecticide Fungicide and Rodenticide Act |
| GRAS      | Generally recognized as safe                      |
| HDOA      | Hawaii Department of Agriculture                  |
| U. S. EPA | United States Environmental Protection Agency     |
| USFWS     | United States Fish and Wildlife Service           |

## Introduction

Two species of *Eleutherodactylus* frogs native to Caribbean areas have established populations in Hawaii, presumably first through introductions in horticultural material and secondarily through inadvertent local transport or intentional anthropogenic movement to new areas (Kraus et al. 1999). Several hundred frog populations have been identified on the four largest Hawaiian Islands—Hawaii, Oahu, Maui, and Kauai (Kraus and Campbell 2002; Pitt and Sin 2004; Pitt et al. 2012). Concerns with the establishment of frog populations range across the tourism and real estate industries (disruptive noise from loud frog calls, Kaiser and Burnett 2006; Beard et al. 2009), the landscape and floriculture industries (reduced profits and inter-island or export quarantines, along with the potential for infection of plants with diseases or parasites, Kraus and Campbell 2002; Kaiser and Burnett 2006), conservation of biodiversity (high density frog populations competing for food with endangered native bird species, Kraus et al. 1999), accidental or intentional export of frogs to fragile ecosystems on adjacent islands or mainland areas (Kraus et al. 1999; Campbell and Kraus 2002; Kraus and Campbell 2002), and regulatory changes related to pest control (Beard et al. 2009).

A variety of physical and chemical control methods have been investigated to allow land owners and government agencies to deal with expanding frog populations initially with the goal of eradication (Kraus and Campbell 2002), later with a focus on local problem management (Beard et al. 2009). Physical methods considered include barriers, hot water or vapor heat treatment, hand-capture or trapping, and habitat management (Beard et al. 2009). Chemicals examined included caffeine, hydrated lime, endosulfan, sodium and potassium bicarbonate and citric acid (Pitt and Sin 2004; Pitt et al. 2012). Although all of these chemicals had toxic effects in laboratory trials, only caffeine, hydrated lime (calcium hydroxide), and citric acid were developed as registered pesticides for spray applications (Campbell and Kraus 2002; USEPA 1992; HDOA 2002; Anon. 2005) and only citric acid was pursued to the point of full operational use (Beard et al. 2009). Citric acid is applied as a 16 % spray or foliage drench to frog occupied areas (HDOA 2002; Pitt et al. 2012). The state and counties have developed various cooperative programs to loan commercial size power sprayers to individuals or groups at no cost. A second method, rarely used, is for aerial application using a helicopter and water drop-bucket, generally used to cover larger and more remote natural areas (Tuttle et al. 2008).

The endemic Hawaiian hoary bat (*Lasiurus cinereus semotus*) has iconic status as Hawaii's only native

terrestrial mammal and is listed as endangered by both the state and federal governments (USFWS 1998). Hawaiian hoary bats are nocturnal, insectivorous, and roost solitarily in tree foliage (USFWS 1998). Jacobs (1996) found that Hawaiian hoary bats can be 45 % smaller than their North American counterparts. Males average a mass of 14.1 g while females are larger with an average body mass of 17.9 g. The pregnancy period for Hawaiian hoary bats lasts from April to June, birthing of young occurs in May or June, with lactation taking place from June to August, and post-lactation occurring from September to December (Menard 2001; USFWS 1998).

Hoary bats have been found in most habitats infested by tree frogs; citric acid applied to forests or other natural areas as well as landscaped or settled areas might result in exposure to hoary bats. Daytime applications would be most likely to result in bat exposure while they are in roost trees. Some bat species undergo diurnal torpor and seem in a dormant state and are not readily disturbed by activities occurring in the surrounding environment. Torpor has not been documented in Hawaiian hoary bats and whether bats would react and vacate areas being sprayed has not been studied. Chances of bat exposure to spray operations might also be higher during the summer pupping season when females are less mobile and their young are nonvolant.

Little ecological information is available for the Hawaiian hoary bat subspecies (Shump and Shump 1982, Whitaker and Tomich 1983, Tomich 1986, USFWS 1998, Gorresen et al. 2013). The listing as “endangered” was based on presumed population declines, suspected habitat loss, and other potential threats inferred from mainland studies of other species. However, there have been no definitive studies of the abundance and population status of hoary bats in Hawaii (Jacobs 1999; Gorresen et al. 2013).

Because pesticide use and contaminants are often cited among the factors affecting endangered species and contributing to bat species declines in particular (Clark et al. 1978; Anon. 1991; USFWS 1998), we and our research colleagues were particularly cognizant of such concerns in developing frog control methods. Bats' fur acts as a barrier to the skin, reducing dermal exposure to contaminants. Thus, the main pathway that bats are likely exposed to chemical contaminants is by oral ingestion (Sample et al. 1997), including grooming. We were particularly concerned with this route of exposure since it has been a preferred method of delivering chiropteracides for control of bat populations (Barclay et al. 1980; Mitchell 1986). Whitaker and Tomich (1983) found generalization of the insectivorous diet by the Hawaiian subspecies of this species; Ratcliffe et al. (2003) reported that bats with insectivorous and frugivorous diets acquired taste-aversions to citric acid. Therefore, it is possible that Hawaiian hoary

bats may avoid ingesting citric acid-contaminated water or insects and would not ingest material contaminating fur when grooming.

Citric acid has been listed as a GRAS (generally recognized as safe) non-regulated, minimum risk pesticide and is exempt from the registration requirements under Federal Insecticide Fungicide and Rodenticide Act by the U. S. Environmental Protection Agency (FIFRA section 25b, 40 CFR Section 152.25). Citric acid is easily degraded by micro-organisms in soil, natural waters, and sewage treatment systems (USEPA 1992). Thus, a number of generic data requirements could be waived in developing its use as a frog toxicant. Nonetheless the general public's and regulatory agencies' concerns with pesticide use in Hawaii's fragile environments led us to examine the environmental effects of citric acid use.

Here we report laboratory and field studies related to potential citric acid exposure and toxic effects on Hawaiian hoary bats. Our studies had five objectives: (1) determine toxicity levels of citric acid to bats, (2) determine the quantity of citric acid solution a bat would voluntarily consume, (3) determine effects of spraying citric acid solution on bats, (4) determine the amount of citric acid solution that could be potentially retained by bat fur, and (5) quantify the amount of citric acid Hawaiian hoary bats might encounter in actual ground and aerial spray operations.

## Materials and methods

### Study subjects

Big brown bats (*Eptesicus fuscus*) were used as a surrogate species for the Hawaiian hoary bat. Both species are insectivorous, similar in size (13–20 g) and belong to the family Vespertilionidae (Tomich 1986; Lollar and Schmidt-French 2002). Thirty-six wild big brown bats (26 females, 10 males) were captured locally in Fort Collins, Colorado, between 20 and 30 August 2007. Bats were individually marked with colored wing bands and group-housed in plastic storage containers (6 bats/container) that were lined with fiberglass screen with cotton cloth covering. These transparent containers were 60 cm long, 40 cm wide, and 35 cm high. Bats were acclimated to feeding on meal worms placed ad libitum in a Petri dish in each container; two additional Petri dishes contained drinking water. Group cages were changed for cleaning weekly. Individual bats were randomly assigned to trials and treatment groups. All trials used individually-housed bats in smaller bins (35 cm long, 30 cm wide, and 30 cm high). Animal rooms were maintained at a temperature of 22 °C.

### Oral toxicity

Citric acid (Sigma-Aldrich, St. Louis, MO; CAS Number 77-92-9) was reagent grade (99–100 % purity) and solutions were prepared weight to weight (w/w) rather than the weight/volume to mix spray solutions. We conducted these initial laboratory and oral toxicity trials using 16 % and 8 % citric acid solutions (w/w) to assess toxic effects (equivalent to 14.6 % and 7.3 % by the unadjusted w/v method of preparation). Throughout the paper, to avoid confusion with the operationally used material, we refer to all solutions based on the equivalent w/v concentrations.

We evaluated oral toxicity of citric acid solutions to bats by gavage by two methods: (1) holding the volume constant at 1 ml and adjusting concentration, and (2) holding concentration constant and adjusting volume. The up-and-down method (Bruce 1985) was used, relying on single animal treatment, observation of symptoms for 2 days, then treating a second animal with either a higher or lower dose depending on the previous response. Planned doses were separated on a 1.4 geometric progression dose; doses were then recalculated to accurately reflect actual delivery of the active ingredient. Syringes were prepared by adding citric acid granules, inserting the plunger to the 1 ml mark, adding water through the neck of the syringe via a needle, rocking to dissolve granules, then attaching an 18 gauge, 5.08 cm gavage needle. Only females were used for this trial, since in some species, they are more sensitive to toxic materials (Risipin et al. 2000).

Symptoms of intoxication varied considerably among individual bats. Minor responses observed included trembling, lethargy, hyperactivity, or gagging. Emesis or deaths were more consistent and observable symptoms; deaths or emesis, within 48 h after gavage, were chosen as decision responses to determine whether the dose for the next bat should be lower or higher. Emesis is an adverse physiological reaction that may protect an individual from a toxic substance by expelling it from the body. Bats were observed directly for the first hour after gavage, hourly for the next 6 h, then every 4 h up to 24 h. Periodic observations occurred from 24 to 48 h post-gavage.

We subsequently evaluated toxicity of only 14.6 % (w/v) citric acid solution by oral gavage and adjusted volumes. We used the same methods and geometric progression as in the first trial to determine treatment volumes. Individual doses were calculated proportionately to a 26 g bat (median bat weight) receiving a 0.50 ml (0.019 ml/kg) gavage. The 0.5 ml dose was used as a starting point so the potential maximum volume needed would be less than 5 % (ml/kg). Thus, a 35 g bat would receive 0.67 ml of 14.6 % citric acid solution.

## Consumption

Voluntary consumption of citric acid by bats was evaluated by assigning two bats each (7 female, 1 male) to treatment groups of 2.0, 7.3, or 14.6 % citric acid solution offered in Petri dishes. A control group was offered water in Petri dishes. Petri dishes were refilled with 50 ml of liquid every 24 h for 3 days and the trial was video recorded. Review of video footage suggested that some bats sampled the solution, but did not drink; therefore observations were terminated after 3 days.

We compared bat weights before and after the trial using a paired *t* test.

## Grooming

Effects of citric acid ingested by bats during grooming were examined by spraying bats over their entire body, including wing and tail membranes, with 5 ml of either 14.6 % citric acid solution ( $n = 5$ ) or with 5 ml of distilled water ( $n = 5$ ), then placing them in their individual containers. Behavior was observed for 4 h afterward; food and water were available ad libitum. We recorded qualitative descriptions of observed behaviors.

## Retention

Twenty euthanized bats were used to evaluate citric acid retention in fur after completion of earlier trials, excluding grooming. A paperclip was used to secure skin near the tail; a carcass was then held by the paperclip and the rostrum (to keep the mouth closed) and immersed in a 14.6 % citric acid solution for 2–3 s. Each bat's weight was recorded before dipping in the solution. Carcasses were hung by the paperclips for either 2 or 30 min, then weighed to determine liquid retention.

## Field assessment methods

To determine potential bat exposure at spray sites, we placed bat effigies to facilitate measurement of citric acid absorption. The bat effigies were constructed of absorbent, pre-formed bundles of cotton batting 50 mm in length, utilizing readily available retail tampons (O.B.<sup>®</sup> Super, McNeil-PPC, Inc.) in order to standardize effigy size. To form effigies that approximated Hawaiian hoary bat volume (25 ml, C. Kishinami, B. P. Bishop Museum, pers. comm.), we soaked the tampons in water to obtain full expansion, then placed each effigy in a foil cup and dried in a convection oven at approximately 84 °C for at least 16 h. Immediately after removal, we recorded the masses of the effigies, including the foil cups as tare. Effigies were kept

in the foil containers and stored in a covered plastic container while in transport to and from the treatment sites.

At the ground treatment sites, we deployed 39 effigies at various heights above ground level (from 100 to 500 cm) immediately before spraying of citric acid and 27 effigies in a nearby reference area (from 100 to 300 cm above ground). We similarly placed 25 effigies in the aerial spray area (at heights ranging from 120 to 500 cm above ground) and 5 in a reference area (effigies in the reference area were inadvertently exposed to spray and were discarded). We recovered effigies immediately after spraying and dried them as before for at least 16 h. The mass of each effigy was recorded after drying and compared to the pre-treatment mass. The mean change in mass of effigies from reference plots (0.01 g) was used to correct the mass changes of the treatment effigies. The differences in mass before and after spraying represented the amounts of citric acid absorbed and were attributed to solid citric acid granules recovered on the dried effigies. We compared the amount of solution retained by effigies using a one-way ANOVA.

## Ground-spray application

The ground-based applications were conducted at eight individual spray sites treated during May and June 2006. The sites ranged from residential properties to native forests (range 50–3,035 m<sup>2</sup>). All treatments occurred between 1600 and 2000 hours and were done with a 16 % citric acid solution. Operators used 1.5 kl gasoline-powered tanks with the adjustable nozzles attached to either a 3.8-cm diameter fire hose or standard size garden hose. This method and citric acid concentration were the same as those used by commercial applicators. We measured the amount of solution applied and the area treated. The operator conducting the spraying varied between sites. Spray application rate (kl-ha<sup>-1</sup> of solution) varied greatly between sites. Of the eight sites, four received less than 19 kl-ha<sup>-1</sup> of citric acid solution, two received between 19 and 28 kl-ha<sup>-1</sup>, and two received over 94 kl-ha<sup>-1</sup>.

## Aerial-spray application

The aerial spray applications took place at the Manuka State Park in the Kau District on the island of Hawaii in December 2006. Hawaii State Parks personnel conducted a substantial aerial citric acid spray operation for the control and containment of coqui frogs (*Eleutherodactylus coqui*). Approximately 58 kl of 13 % (w/v) citric acid were applied via helicopter and drop-bucket over 3 days, covering an area of 4.13 ha. We conducted our experiment on the third day of aerial operations, during which 21 kl of citric acid solution were applied to 1.46 ha between 1100 and 1500 hours. The approximate application rate was 14 kl-ha<sup>-1</sup> (1,600 gallons/

acre), delivered from an approximate height of 50 m above ground level.

## Results

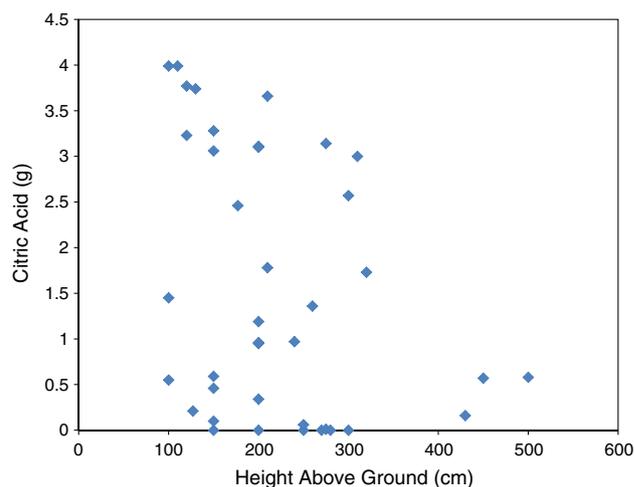
### Oral toxicity

The amount of citric acid considered toxic to the bats, based on the adverse physiological reaction of emesis, was within the range of 542 and 759 mg/kg. At the highest dose delivered, 7,361 mg/kg, death of the bat occurred within 4 min. Emesis occurred in one of three bats dosed at 759 mg/kg. No effects were observed at 542 mg/kg, the lowest dose delivered. Most emesis occurred within the first hour after gavage, sometimes as soon as 3 min. The volume of 14.6 % citric acid solution estimated as toxic to the bats by ingestion, based on emesis, was between 0.14 and 0.26 ml. The first and highest dose, 0.57 ml, resulted in death of the bat within 4 min. The lowest dose at 0.10 ml did not result in emesis.

### Grooming, citric acid consumption, and liquid retention

In grooming trials, bats treated with water were observed shaking (to release water from their fur) and grooming, including licking and scratching or combing with back feet or thumbs. Most bats sprayed with citric acid solution removed fur with their feet, probably because it became sticky and matted. Sticky fur that was pulled out was generally removed from the feet by licking. Some bats ate a few meal worms during the 4-h observation, but none were observed to drink. Emesis was not observed for any of the bats in these trials. As bats dried, fur appeared either combed or became thinner due to removal. None of the bats sprayed with distilled water were observed removing fur. Bats were monitored for a week following treatment; all survived in apparent good health. Additionally, bats gained a small amount of weight (about 1.5 g) over the course of the trial. The starting average bat weight was 24.96 g ( $\pm 3.10$  SD) while the average end bat weight was 26.53 g ( $\pm 3.97$  SD). The starting and end weights were not significantly different ( $t = 1.25$ ,  $P = 0.2225$ ).

The procedure used to attempt to estimate direct voluntary consumption of citric acid solutions was flawed in that animals became wet from walking in the open Petri dishes used to present the liquid. Review of video footage suggested that none of the bats drank the solution, although there were a few questionable instances. Results and observations generally paralleled those for the test on grooming in that bats appeared to avoid consumption of citric acid. Some bats, upon apparently licking the solution,



**Fig. 1** Citric acid residues recovered from Hawaiian hoary bat effigies at ground application sites

heaved their head backward away from the solution and often quickly left the Petri dishes.

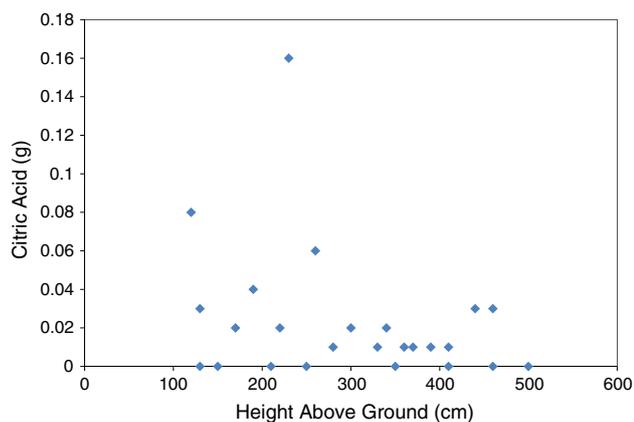
In examining retention of the citric acid solutions in bat fur, we found that bat carcasses retained a mean of 1.06 g ( $\pm 0.20$  SD) of citric acid solution after 2 min of drainage ( $n = 10$ ) and 0.45 g ( $\pm 0.11$  SD) of citric acid solution after 30 min ( $n = 10$ ).

### Ground spray application

The average citric acid load of all treatment bat effigies was 1.54 g ( $\pm 1.43$  g) (dry mass), but ranged widely from 0 to 3.99 g. There appeared to be a decreasing trend in the amount of citric acid absorbed as the placement height increased but the differences were not significant among low, moderate, and high height classes ( $F = 2.52$ ,  $P = 0.0948$ ) (Fig. 1). When grouped by height, the effigies between 100 and 150 cm above ground level absorbed on average the most citric acid (2.03 g,  $n = 14$ ). The average citric acid load of effigies placed between 151 and 250 cm above ground was 1.43 g ( $n = 13$ ). Effigies placed higher than 250 cm above ground retained the least citric acid (1.09 g,  $n = 12$ ). When grouped by volume of citric acid solution applied, effigies at sites receiving  $< 28$  kl-ha<sup>-1</sup> of citric acid solution had an average citric acid load of 0.90 g ( $n = 24$ ). Effigies at sites sprayed with more than 93.5 kl-ha<sup>-1</sup> averaged 2.75 g ( $n = 13$ ). This suggested a positive linear relationship between citric acid load and application rate.

### Aerial spray application

The mean citric acid load of the treatment effigies was very low (0.02 g), although values ranged from 0 to 0.16 g (standard deviation 0.03). Here also, there was no apparent relationship between height above ground and citric acid

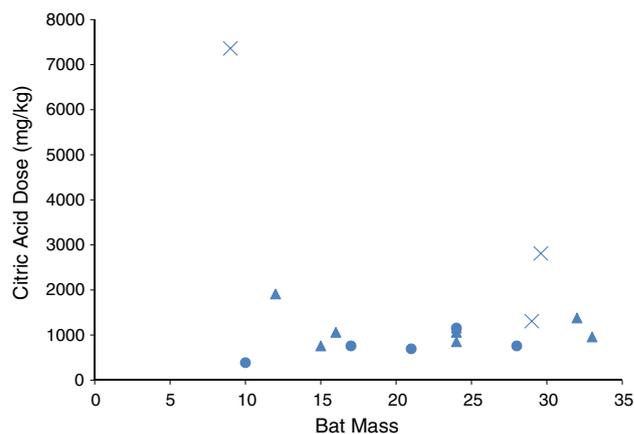


**Fig. 2** Citric acid residues recovered from Hawaiian hoary bat effigies at aerial application sites

retained (Fig. 2). When grouped by height, the effigies placed between 100 and 199 cm above ground retained an average of 0.03 g of citric acid ( $n = 6$ ). The average citric acid load of effigies placed between 200 and 299 cm above ground was 0.04 g ( $n = 6$ ). Effigies between 300 and 399 cm above ground received an average of 0.01 g of citric acid ( $n = 7$ ). Those 400 cm above ground or higher received an average of 0.01 g of citric acid ( $n = 6$ ). The amount of citric acid retained by bat effigies did not vary significantly across height classes ( $F = 0.76$ ,  $P = 0.4810$ ). Spray volumes per unit area were substantially lower in the aerial applications compared with those at ground application sites (about 14 vs. 39  $\text{kl}\cdot\text{ha}^{-1}$ ), and retained citric acid was, on average, about 75 times less.

## Discussion

Spray applications of citric acid are quick and effective at killing invasive coqui frogs and controlling infestations, but the potential for exposure to Hawaiian hoary bats and of any conservation consequences have not been previously addressed. In oral gavage trials, bat toxicity gauged by death or emesis associated with administration of the 14.6 % citric acid solutions used in frog control occurred between 0.14 and 0.26 ml, equivalent to 852–1,309 mg/kg of citric acid (based on a 26 g bat) or 20–38 mg of citric acid (Fig. 3). Further, using the minimum emetic dose or maximum tolerated dose provides a more conservative estimate of toxicity than using only death as an end point. The lowest dose that caused bat mortality was 2,811 mg/kg. Bats did not voluntarily consume measureable amounts of citric acid. Our video footage taken during treatment trials suggested bats could detect the adulterated solutions and probably would not drink citric acid contaminated



**Fig. 3** The fate of bats dosed with a citric acid solution from trials with either a constant volume (0.5 ml) or constant solution concentration (16 %). Bat response to each dose is designated as *closed circle* for bats with no observable effects, *closed triangle* where emesis was recorded, and *times* for bats that died

water pooled in operational areas. This interpretation is consistent with Ratcliffe's (2003) findings wherein bats with generalized diets acquired taste-aversions to citric acid.

Bats sprayed with 5 ml of citric acid solution showed no evidence of intoxication. Five millilitres of 16 % citric acid solution (w/w) would contain about 728 mg of citric acid, but 2 min after application only 155 mg of citric acid remained on the fur. However, bats physically removed the citric-acid coated fur with their feet; then licked the fur off of their feet. They did not ingest amounts of citric acid during this process that caused observable effects. In areas with frequent precipitation, rain could rinse much of the citric acid from bat fur, whether the bat was roosting or flying, rendering citric acid residues a non-concern.

The citric acid loads measured for bat effigies at ground-based application sites were highly variable but appeared to generally decrease with increasing height above ground level. At each site there were different spray operators applying the citric acid solution. Each spray operator had a different spray technique, influencing which areas of the foliage received the most citric acid solution. In all cases, the operators were focused on drenching the ground and understory. When the spray nozzles were pointed upward into the foliage, the spray streams tended to fan, increasing the likelihood of interception by foliage. Vegetation type could be a further determining factor of how much citric acid solution would penetrate through the foliage to roosting bats. It appears from our results, with higher variability of citric acid retention on effigies nearer the ground and from our observations of the ground spray application procedures, that bats would encounter less

citric acid solution the higher they were roosting above ground. Although bats have been observed roosting close to the ground, Gorresen et al. (2013) reported that day-roosts generally occur in trees greater than five meters crown height. Our results were also consistent with the presumption that when greater volumes of solution were applied, the chances of bat exposure increased. The citric acid loads for all bat effigies in the aerial application were minimal, suggesting that bats would have very low risk for citric acid exposure during such operations. Furthermore, the amount of citric acid absorbed by the effigies greatly exceeded the potential amount that a bat's fur could absorb as observed in laboratory trials.

While citric acid is recognized as a mild skin irritant (rabbit-24 h, Sigma-Aldrich MSDS 4.0, 2010, <http://www.sigmaaldrich.com/catalog/Display/MSDSContent.do>, accessed July 7, 2011), dermal toxicity is not indicated. Furthermore, the fur and hair of mammals affords protection from direct skin contact, reducing dermal exposure to contaminants; thus the main pathway of mammal exposure to chemical contaminants is by oral ingestion (Sample et al. 1997). Therefore, the greatest concern would be that bats sprayed with citric acid will inadvertently ingest it while grooming. However, no toxic effects resulting from grooming contaminated fur were observed in this study. Rather, the primary potential impact identified in this study is from hair loss during grooming. The effect of hair loss on thermoregulation in the tropics and how long this effect would last is unknown. Additionally, the extra grooming may consume excessive energy and weaken the animal, and/or reduce the time spent foraging. The effect of hair loss may be greater on bats in nontropical areas and bats exposed to repeated citric acid applications may be at greater risk from hair loss or excessive grooming.

While the risk of exposure to citric acid at levels shown to cause signs of toxicity is very low for both ground and aerial spraying, there is still the potential for other, non-toxic, impacts to bats, so exposure to spray volumes high enough to coat a bat's fur should be avoided. This scenario is most likely to occur during ground-based treatments, when the applied volume is highest. To minimize the potential for exposure to bats, citric acid treatment planning should take into account the time of year to avoid exposure to lactating females and their young, set maximum spray heights, and train applicators to avoid spraying any area with excessive volume. We conclude that current frog control operations with citric acid, as already approved and conducted, pose little toxic threat to Hawaiian hoary bats because individuals would likely not be exposed to toxic doses of citric acid if inadvertently sprayed.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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