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PROBABILISTIC RISK ASSESSMENT FOR SNAILS, SLUGS, AND ENDANGERED HONEYCREEPERS IN DIPHACINONE RODENTICIDE BAITED AREAS ON HAWAII, USA

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Abstract—Three probabilistic models were developed for characterizing the risk of mortality and subacute coagulopathy to Poouli, an endangered nontarget avian species, in broadcast diphacinone-baited areas on Hawaii, USA. For single-day exposure, the risk of Poouli mortality approaches 0. For 5-d exposure, the mean probability of mortality increased to 3% for adult and 8% for juvenile Poouli populations. For Poouli that consume snails containing diphacinone residues for 14 d, the model predicted increased levels of coagulopathy for 0.42 and 11% of adult and juvenile Poouli populations, respectively. Worst-case deterministic risk characterizations predicted acceptable levels of risk for nonthreatened or endangered species such as northern bobwhite quail and mallards. Also, no acute toxicity was noted for snails and slugs that feed on diphacinone baits.

Keywords—Probabilistic Risk Poouli Diphacinone Rodenticide

INTRODUCTION

Introduced rodent species can negatively impact native ecosystems. For example, rats have contributed to the extinction of indigenous flora and fauna on Hawaii, USA, as well as other islands [1]. The control of rodent pests (rats, mice, ground squirrels, opossum) in agricultural and urban environments relies primarily on the use of rodenticides. Warfarin, a widely used first-generation anticoagulant rodenticide, largely has been replaced by the more toxic rodenticides such as diphacinone, chlorophacinone, and brodifacoum [2,3]. For example, the acute oral median lethal dose (LD50) for rats (*Rattus* sp.) is 59 mg/kg for warfarin and 2 mg/kg for diphacinone [4]. Current annual usage of rodenticides in the United States is approximately 3,000 lb of active ingredients (6 million lb of rodenticide baits) [5]. In remote areas, broadcast application of rodenticide baits such as diphacinone have been shown effectively to control rat populations [6]. In humid environments such as Hawaii, all-weather rodenticide baits (grain fortified at 0.005% weight/weight [w/w] diphacinone encapsulated in wax or pressed with oil) commonly are used.

When considering the use of rodenticides to control destructive introduced rodent species, risks to native species also must be considered. For example, gastropods (snails and slugs) have been observed to consume rodenticide baits in bait stations and on forest floors [7]. It is likely that lipophilic rodenticides such as diphacinone subsequently would be absorbed and retained by the gastropods. It is plausible that birds may be exposed to rodenticides through the consumption of diphacinone-laden gastropods.

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Invertebrate exposure to rodenticides

Following the aerial distribution of Brodifacoum rodenticide baits on Red Mercury Island and Coppermine Island, New Zealand, a variety of invertebrates, including snails and slugs, were collected on and around the baits [8]. In another study, Spurr and Drew [9] monitored invertebrates feeding on four different types of rodenticide bait matrices that were placed on the New Zealand forest floor. All bait types were consumed by terrestrial invertebrates [9].

Similar results were reported by Dunlevy et al. [7]. Following broadcast application of placebo rodenticide baits in Hawaiian forests, 21 species of invertebrates were observed on the baits. Although ants were the most abundant species, snails and slugs represented 27% of the observed invertebrates. *Deroceras laeve* (yellow slug) accounted for more than half of the gastropods observed on the baits.

Anticoagulant rodenticides generally are less toxic to invertebrates than to mammals or birds. For example, 0.002% brodifacoum baits are extremely toxic to most rodent and raptor species. However, when fed to crabs for several days, no toxicity was observed [10]. With respect to pesticide adsorption, disposition and toxicity, crabs are more similar to gastropods and insects than mammals or birds [11]. This suggests that invertebrates may be able to feed on rodenticide baits or carcasses for extended periods without suffering acute toxicosis. This would permit invertebrates to ingest and retain significant quantities of anticoagulant rodenticides. However, because Hawaii contains endangered species of snails, it would be prudent to examine the potential toxicity of diphacinone to snails and slugs before beginning widespread distribution of diphacinone baits. Quantification of diphacinone residues in snails and slugs that had consumed diphacinone rodenticide

baits could be used to estimate diphacinone ingestion in birds that feed on gastropods.

Rodenticide risks to invertebrate-eating birds

Past studies have evaluated avian risks posed by rodenticide use. Most have examined the risk to predatory or scavenging species. However, a few studies have suggested a link between rodenticide use and risk to nontarget birds that consume invertebrates as a significant portion of their diet.

Many passerine and duck species primarily consume invertebrates. Magpies, hawks, and seagulls routinely consume insects, snails, and slugs [12]. Following baiting with 0.005% brodifacoum rodenticide baits, Rammel et al. [13] collected carcasses of nontarget animals for as long as 28 d postbaiting. Nontarget fatalities included ducks, seagulls, hawks, magpies, and passerines. In all the nontarget species collected, the highest brodifacoum levels were observed in the liver, followed by fat and muscle. The highest mean liver concentration (8.1 parts per million [ppm]) was detected in a passerine bird (species unreported). The common dietary link between all the species of poisoned birds detected in this study was invertebrates. These birds likely were exposed to brodifacoum through the consumption of brodifacoum-containing invertebrates.

Following a rodenticide (sodium monofluoroacetate)-baiting program in New Zealand, robin populations decreased by approximately 50% in baited areas compared to populations in unbaited areas. Autopsy of freshly dead robins revealed fragments of invertebrate exoskeletons in the gizzard. None of the autopsied robins that were found dead following the baiting program contained remnants of rodenticide bait in their digestive tracts. Other invertebrate-consuming species found dead following the baiting program include the tomit, gray warbler, and rifleman. None of the gastro-intestinal tracts contained rodenticide baits [14], suggesting that secondary exposure via consumption of invertebrates may have been responsible for delivering lethal doses of rodenticides to these birds.

Following a brodifacoum-baiting program in New Zealand, significant population decreases were observed for the robin (50%), weka (100%), kaka (20%), and morepork owl (25%) [15]. The diet of the robin consists almost entirely of insects; the weka diet consists primarily of insects, snails, and slugs; the kaka consumes insects and fruit; and the morepork primarily consumes large insects, snails, and slugs [12]. Again, the primary link between all these nontarget fatalities is the consumption of significant quantities of invertebrates.

In another study, little spotted kiwi populations decreased by 10% following baiting with a 2% brodifacoum bait. The diet of the kiwi consists exclusively of small invertebrates such as the larval stages of insects and slugs [16]. The results of this study reinforce the hypothesis that rodenticide poisoning may be mediated by invertebrates.

Magnitude and persistence of rodenticide residues

The persistence and potency of anticoagulant rodenticides suggests that the risk of accidental poisoning of nontarget wildlife is greater than that associated with less persistent widely used modern organophosphate and pyrethroid insecticides and triazine and glyphosate herbicides [16]. Because anticoagulant rodenticide residues can remain in animal tissues for more than 8 months [17], birds feeding on rodenticide-containing invertebrates may be accumulating a toxic dose over an extended period of time.

The probability of such a scenario is reinforced in a review of over 200 published references (encompassing 62 pest species) that concludes that repeated exposure to anticoagulant rodenticides on successive days did not decrease the total dose needed for acute toxicity [18]. Essentially, the dose accumulated over multiple days is additive. Given the long persistence of rodenticide residues in exposed animals, this suggests that secondary poisoning of nontarget wildlife may result from the repeated consumption of prey (including invertebrates) containing low levels of these rodenticides. In a study of nontarget fatalities associated with successive baiting with anticoagulant rodenticides (one month apart), a significant increase in nontarget poisoning was documented after the second baiting [15].

Anticoagulant rodenticide toxicity to birds

The lethal dose of rodenticides to most native birds is unknown [15]. However, given the persistence of rodenticides in prey and the ability for nontarget species to accumulate toxic doses over an extended period of time, many species of birds may be at significant risk with respect to anticoagulant rodenticide use. In a review of nearly 50 secondary poisoning studies with rodenticides, Joermann [19] concluded that anticoagulant rodenticides are acutely toxic. In a seven-year survey of nontarget wildlife poisonings in New York State, USA, anticoagulant rodenticide (diphacinone, chlorophacinone, brodifacoum)-poisoned birds accounted for more than half of the wildlife fatalities [20].

Subacute effects

Savarie et al. [21] examined the effects of secondary diphacinone exposure to birds. In this study, golden eagles were fed 454 g (1 lb) of sheep tissue containing average incurred diphacinone residues of 2.7 ppm. The eagles were offered 454 g (1 lb) of this sheep tissue per day for 5 (4 birds) or 10 (3 birds) consecutive days. Based on consumption, the mean diphacinone doses for eagles were 0.17 mg/kg/d (0.87 mg/kg total) or 0.16 mg/kg/d (1.16 mg/kg total) for the 5 and 10-d exposure groups, respectively. Although no acute toxicity was noted in any of the eagles, prolonged prothrombin clotting times were noted for all diphacinone-exposed treatment groups.

Although rodenticides offer many potential benefits to agriculture and ecosystem restoration efforts, potential risks to nontarget wildlife must be considered before wide-scale rodenticide baiting programs should be initiated. The purpose of this study was to assess such risks with respect to a proposed diphacinone broadcast–baiting program for the control of introduced rats on Hawaii. Diphacinone residues were quantified in snails and slugs that had fed on diphacinone-containing rodenticide baits for 7 d in a laboratory setting or had been collected on or near diphacinone rodenticide baits during a diphacinone-baiting program to control introduced rats on Hawaii. These data were used to estimate potential diphacinone exposure and associated risks for birds potentially consuming gastropods.

MATERIALS AND METHODS

Animal procurement

For the laboratory exposure study, slugs (*Limax maximus* and *Deroceras laeve*) and snails (*Oxychilus* spp.) were collected from Hawaii Volcanoes National Park. Animals for con-

trol analyses, analytical method development, and laboratory-exposure tests were collected from areas with no history of diphacinone use. Protective plastic bait stations were placed on the ground at 5-m intervals along trails and were maintained at high humidity with Perlite and water. A dilute solution of liquid manure and fish emulsion was added as a scent lure to attract snails and slugs. Snails and slugs were removed from the stations daily. Slugs and snails also were collected by searching in leaf litter and other appropriate areas.

To permit the determination of residues from animals exposed to diphacinone under field conditions, snails and slugs were collected from areas where diphacinone rodenticides currently were being used. For the field-exposure study, invertebrates were collected on or within 1 m of rodenticide baits.

Animal maintenance

For the laboratory-exposure study, snail and slugs were maintained at the U.S. Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services National Wildlife Research Center Hawaii Field Station (Hilo, HI, USA). Animals were segregated by species. *Limax maximus* (1–3 individuals), *Deroceras laeve* (3–6 individuals), and *Oxychilus* spp. (≈45 individuals) randomly were assigned to treatment and control containers. All individuals within each container were weighed to determine biomass per container. Gastropod biomass was recorded before the beginning and at the end of each experimental trial.

Containers consisted of 4-inch diameter styrofoam® containers with plastic lids. Containers were 5-cm deep and were covered with plastic lids containing 10 holes (1-mm diameter) to provide ventilation for snails and slugs. To provide moisture for the test animals, paper towels moistened with distilled water were placed on the bottom of each container. Snails and slugs were maintained on Purina® (St. Louis, MO, USA) laboratory rodent chow ad libitum under an approximately equal light:dark cycle at 21°C.

Laboratory diphacinone exposure

A total of 45 (n = 45) gastropod samples (*Limax maximus* [n = 15], *Deroceras laeve* [n = 15], and *Oxychilus* spp. [n = 15]) were offered HACCO Ramik® Green (Madison, WI, USA; fish-flavored, weather-resistant pelletized rodenticide bait containing 0.005% rodenticide bait) ad libitum for 7 consecutive days. Rodent chow was not provided during the test period. A control group of five samples of each species (n = 15) was offered rodent chow instead of Ramik Green. For each species, samples containing approximately 3.0 g of gastropod tissue (*Limax maximus* ≈ 1 individual, *Deroceras laeve* ≈ 9 individuals, *Oxychilus* spp. ≈ 45 individuals per sample) were collected immediately after the 7-d feeding period (n = 5), 24 h after the bait was removed (n = 5), and 7 d after the bait was removed (n = 5).

Frozen samples were shipped to the National Wildlife Research Center (NWRC) analytical chemistry laboratories (Fort Collins, Colorado, USA) for diphacinone residue analyses. Samples were shipped with dry ice to ensure that samples remained frozen during shipping.

Chemical analysis

The NWRC Analytical Chemistry Method 105 (Determination of diphacinone residues in snails and slugs) was developed and validated by the NWRC Analytical Chemistry Project [22]. This method was used to quantify diphacinone residues in snail and slug whole body tissues. Diphacinone residues in control, laboratory-exposed, and field-exposed gastropods were quantified by reversed-phase ion-pair high-performance liquid chromatography. The samples were frozen in liquid nitrogen and homogenized in a Spex (Metuchen, NJ, USA) Centiprep 6850 freezer mill. A 0.5-g aliquot of the homogenized tissue was mixed with 5 g sodium sulfate and extracted in triplicate with 10 ml acidified chloroform:acetone (1:1). The extracts were pooled and evaporated to dryness at 60°C under a gentle stream of nitrogen. The residue was reconstituted in 2 ml chloroform and 3 ml hexane and subsequently cleaned up via elution through a solid-phase extraction column containing 500 mg aminopropyl sorbent. The solid-phase extraction column was rinsed with 3 ml hexane:chloroform (2:1) and 3 ml chloroform. Diphacinone was recovered from the column by elution with 10 ml of 4 mM methanolic tetrabutylammonium phosphate. This eluate was reduced to dryness by evaporation under nitrogen and redissolved in 1.0 ml 60:40 methanol:water containing 5 mM tetrabutylammonium phosphate. Diphacinone was separated by reversed-phase ion-pair high-performance liquid chromatography using a C18 column and a pH 8.5 mM tetrabutylammonium:50 mM dihydrogen phosphate in methanol:water gradient mobile phase. Diphacinone was quantified by ultraviolet detection (325 nm) against an external standard calibration curve.

Statistical analysis

Diphacinone residue data and residuals were examined for normality by using the Shapiro-Wilk test in PROC UNIVARIATE (SAS, Gary, NC). The variance of the residuals was examined visually by plotting the residuals versus predicted values. Diphacinone levels in gastropod species were compared via two-way analysis of variance with residue concentration as the response and species, time, and species·time as the independent variables. Fisher's least-significant difference test was used for multiple comparison means [23].

Deterministic risk quotients

Comparison of risk quotients to levels of concern is a screening approach used by the U.S. Environmental Protection Agency (U.S. EPA) for evaluating worst-case potential hazards to nontarget species [24]. For this study, risk quotients were calculated by dividing exposure (upper 95th percentile of *Deroceral* diphacinone concentrations from laboratory-feeding study = 4.93 ppm) by the median lethal concentration (LC50) for potentially exposed nontarget species. These LC50 estimates were generated from dose versus mortality experiments conducted with juvenile birds. In diphacinone-feeding studies conducted by Shirazi et al. [25], adult northern bobwhite quail LC50 values were 2.5 times greater than juvenile values. Based on these findings, diphacinone LC50 values for juvenile northern bobwhite quail and mallards were multiplied by 2.5 to give LC50 estimates for adult birds of the same species. The LC50 values for adult and juvenile Poouli were estimated by dividing the appropriate mallard LC50 value by 38.5, the interspecies range of toxicity values for the anticoagulant rodenticide Brodifacoum. These risk quotients were compared with the 0.5 level of concern for nontarget species or 0.1 level of concern for threatened or endangered species, as appropriate for the species of consideration [24].

Probabilistic model

Probabilistic models were constructed to apply single-day toxicity and 5-d dietary exposure toxicity data to the risk as-

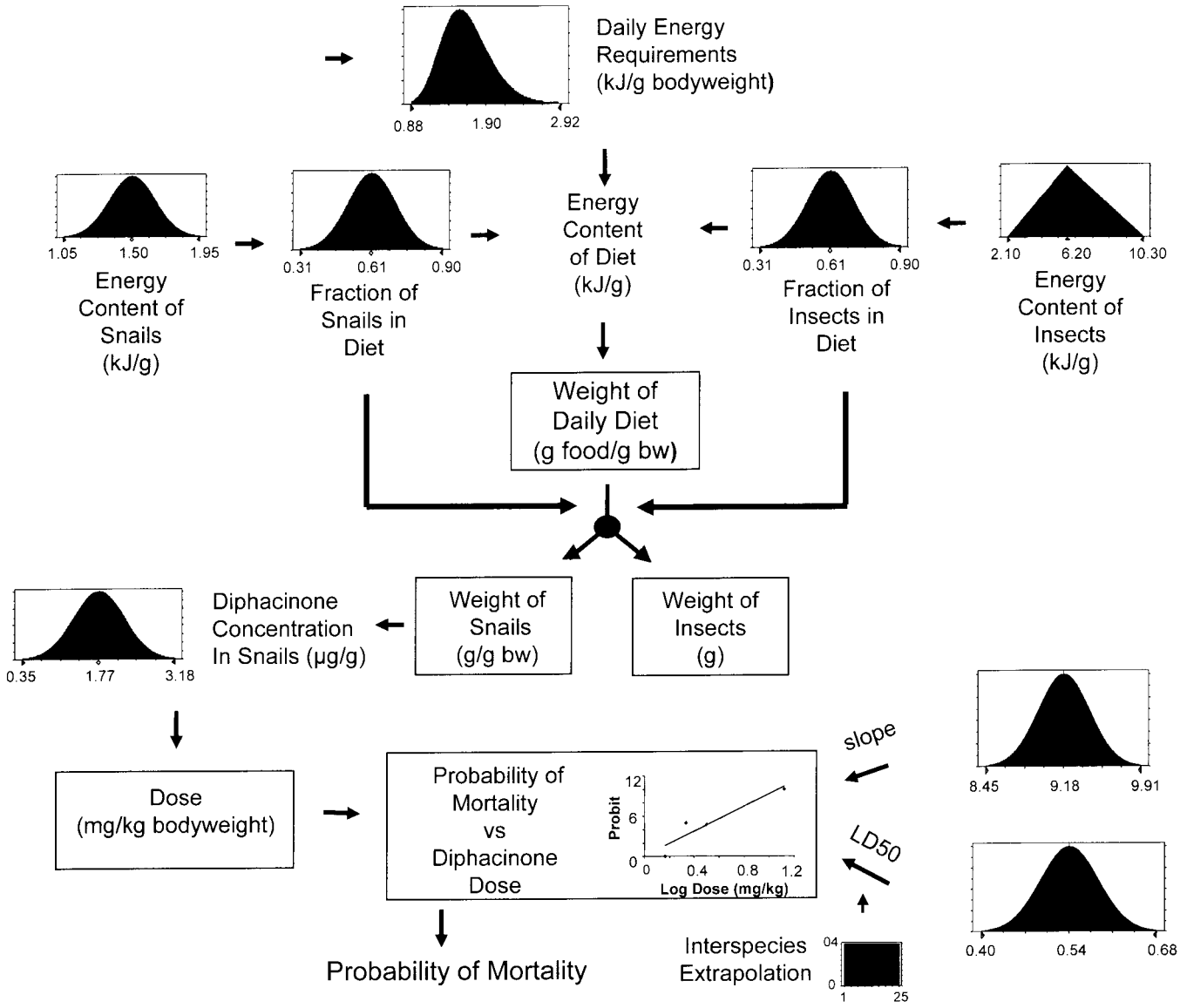

Fig. 1. Probabilistic model based on single-day acute exposure. LD50 = median lethal dose; bw = bodyweight.

sessment using Crystal Ball (Decisioneering, Denver, CO, USA). Each iteration of the model simulated consumption and diphacinone exposure and then calculated the risk of mortality for an individual bird. Each iteration of the single-day exposure model was initiated by random selection of a daily energy requirement from a normal distribution of energy needs as calculated by Nagy et al. [26] for a 32-g adult and 5-g juvenile passerine (Fig. 1). The fraction of snails and insects in the daily diet of each bird was estimated by random selection of normally distributed values based on previously reported contents of Poouli gastrointestinal tract [27]. Energy content distributions for snails and insects (beetles) [28,29] were sampled and multiplied by the dietary fraction of snails and insects to yield the overall energy content for the daily diet. This daily energy requirement was divided by energy content to yield the weight of the daily diet. This was multiplied by the fraction of snails in the diet to yield the weight of snails consumed. The weight of snails consumed was multiplied by a randomly selected value from the distribution of snail diphacinone-residue values to yield the daily diphacinone dose (µg diphacinone/g body weight). This dose was regressed against a dose versus probability mortality curve constructed for each bird to yield a probability of mortality. The dose versus probability curve was constructed from randomly sampled values from normal distributions of slope and log median dose (LD50) values for adult quail receiving a single dose of diphacinone [30–32]. Because the interspecies range of reported rodenticide LD50 values differ by a factor of 38.5, the estimate quail LD50 values were extrapolated to Poouli applicable values following division by a randomly selected value between 1 and 38.5. Using the Crystal Ball (Decisioneering) 2D function, 95% confidence intervals (CIs) for mortality predictions were calculated by running 200 uncertainty trials consisting of 100 variability trials each. The fraction of snails in the diet and the diphacinone content of snails were categorized as variability with the remaining assumptions categorized as uncertainty.

The 5-d exposure model was initiated by multiplying randomly selected values from the normal distributions of the fraction of snails in the diet and concentration of diphacinone residues in diphacinone-exposed snails to yield a dietary di-

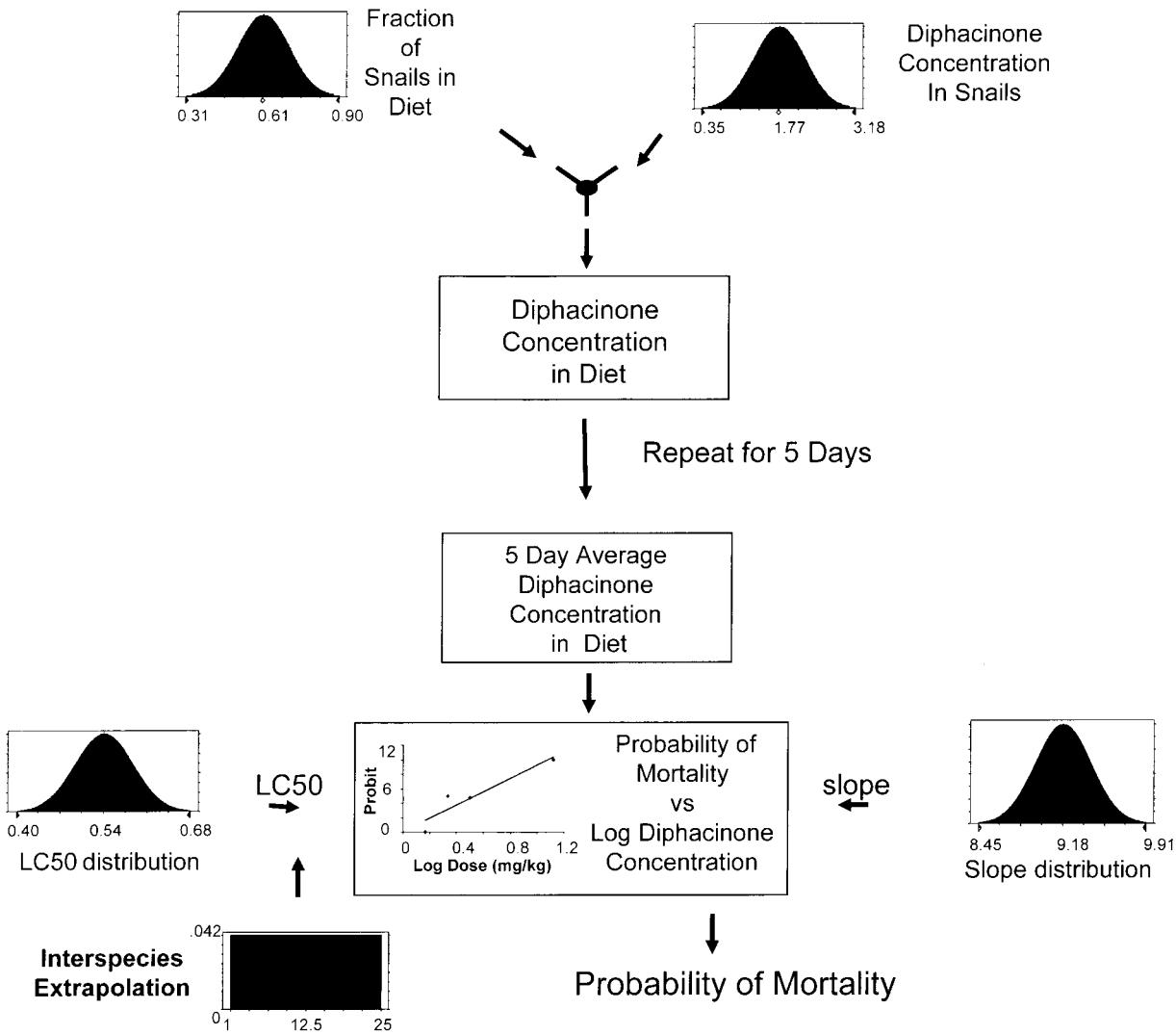

Fig. 2. Probabilistic model based on 5-d dietary exposure toxicity data. LC50 = median lethal concentration.

phacinone concentration (Fig. 2). This concentration was regressed against the diphacinone dietary concentration versus probability of mortality curve for juvenile mallards fed diphacinone-fortified diets for 5 consecutive days [33]. For estimation of adult mortality, these LC50 values were extrapolated to adult LC50 values following multiplication by 2.5 [25]. This age-related differential in LC50 likely stems from juvenile versus adult differences in food consumption rates (g food/g body wt). The LC50 values were extrapolated to estimate Poouli LC50 values following division by a randomly selected value between 1 and 38.5. Using the Crystal Ball 2D function, 95% CIs for mortality predictions were calculated by running 200 uncertainty trials consisting of 100 variability trials each. The fraction of snails in the diet and the diphacinone content of snails were categorized as variability with the remaining assumptions categorized as uncertainty.

The probability of nontarget subacute effects was estimated using the single-day exposure probabilistic model with the following modification: For each iteration, a diphacinone dose was calculated for 14 consecutive days. A risk quotient then was calculated by dividing the average diphacinone dose by the LD50 for each bird. A distribution of risk quotients was generated for 10,000 birds (Fig. 3). These risk quotients were compared to a 0.017 level of concern [34].

RESULTS AND DISCUSSION

Diphacinone toxicity to snails and slugs

At the end of the 7-d posttreatment period, all control and diphacinone-exposed snails and slugs were viable. This indicates that the acute primary toxicity of diphacinone to snails and slugs feeding on diphacinone rodenticide baits is minimal.

Diphacinone residues in snails and slugs

Diphacinone residues in laboratory diphacinone–exposed snail and slug tissue of the three species analyzed ranged from <limit of detection to 4.00 mg/kg (Table 1). The diphacinone residue data were normally distributed ($p = 0.356$); the residuals were normally distributed ($p = 0.637$) and homogeneous. The analysis of variance indicated that the effect of species was highly significant ($p < 0.0001$), although time ($p = 0.2493$) and species·time ($p = 0.443$) were not significant. Because the effect of species was highly significant, mean residue values for each species were compared (Table 1). For these data, the Fisher's test indicated a least significant dif-

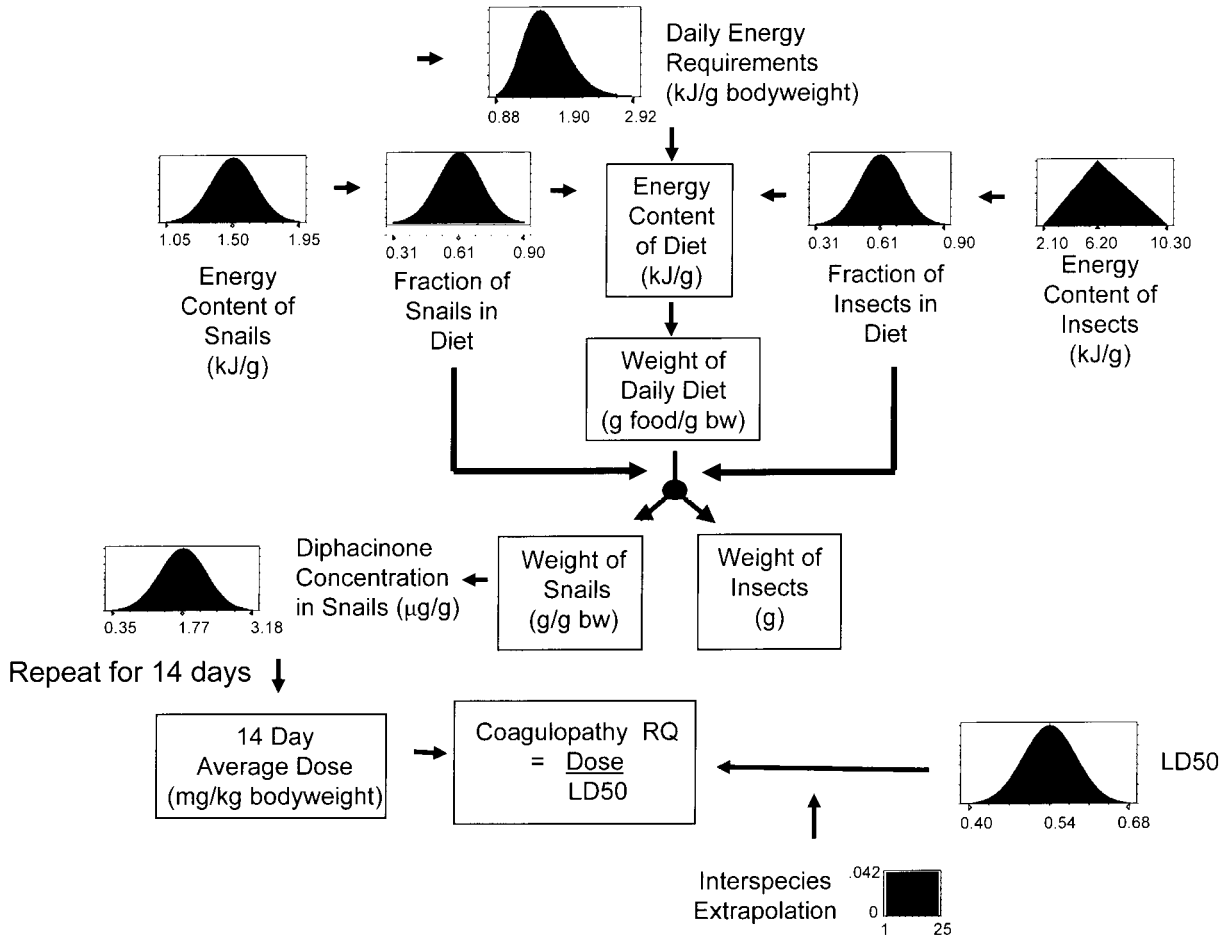

Fig. 3. Probabilistic model for prediction of coagulopathy based on 14-d exposure data. RQ = risk quotient; LD50 = median lethal concentration; bw = bodyweight.

ference of 0.4813. Based on this value, the mean residue for each species of gastropod was significantly different. The diphacinone residues in *Deroceras* contained the highest residues and *Limax* contained the lowest. The magnitude of residues in *Oxychilus*, the only snail tested, were midway between the two slug species.

For each species, the diphacinone residues in the gastropods did not change significantly between the first sampling interval (immediately following 7-d exposure period) and the last sampling interval (7 d postexposure). This conclusion is confirmed by visual inspection of the mean residue (± standard deviation) versus sampling period data presented in Figure 4; for each species, the error bars overlap for all sampling periods. Based on these results, data from all sampling periods were pooled for exposure/risk assessment. The fact that diphacinone concentrations in gastropods did not decline for 7 d postexposure is consistent with the observation that anticoagulant rodenticide poisoning is somewhat cumulative; doses acquired over several days are excreted very slowly.

The magnitude of residues observed in field-collected gastropods was less than half of that observed in the laboratory study. This was due to the fact that, under field conditions,

Table 1. Residues of diphacinone in laboratory-exposed snail and slug tissues

Species	Range of residue concn. (ppm)[a]	Mean residue concn. (ppm)[b]	95% Percentile residue concn. (ppm)	*t* Tests (LSD)[c]
Snails				
Oxychilus spp. *n* = 15	1.06–2.91	1.77	2.79	A
Slugs				
Limax maximus n = 19	<MLOD–2.26	0.806	2.08	B
Deroceral leave n = 37	1.63–5.01	2.64	4.93	C

[a] ppm = parts per million.
[b] To calculate the mean residue for samples reported as <MLOD (method limit of detection), the MLOD was used as the value for these samples.
[c] LSD = least significant difference; means with the same letter are not significantly different (p = 0.005).

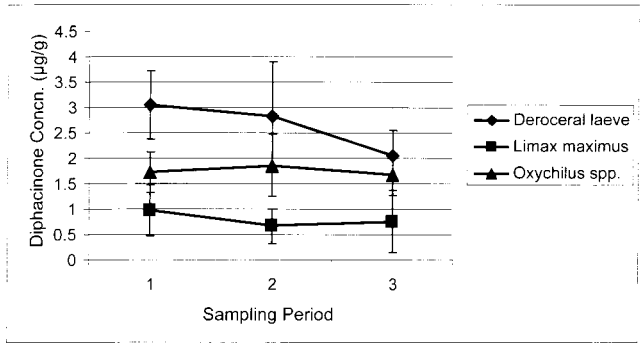

Fig. 4. Diphacinone residues versus sampling period.

gastropods have a variety of potential food sources. However, in the laboratory feeding trials, the only food source available was the diphacinone baits. Contrary to the laboratory study, higher levels of diphacinone were observed in *Oxychilus* snails than in any of the slug species; mean residue values ranged from 0.69 ppm for *Oxychilus* to 0.23 ppm for *Deroceral leave* (Table 2). This suggests that, under field conditions, snails probably spend more time consuming the baits than do slugs.

Secondary exposure assessment

Deterministic risk assessment. Hawaiian birds could be exposed to diphacinone in baiting areas via consumption of gastropods containing diphacinone residues. Both quail and mallard inhabit the Hawaiian Islands and consume invertebrates including gastropods [35,36]. Potential daily exposure can be determined via the widely used approach (U.S. EPA Ecological Committee on Federal Insecticide, Fungicide, and Rodenticide Act Risk-Assessment Methods Terrestrial Draft Report 1999; http://www.epa.gov/ecotox):

$$\text{dietary diphacinone concentration (mg diphacinone/kg food or ppm)} = PD \cdot C \cdot PT$$

The *PD* is the proportion of diet consisting of gastropods (unitless), *C* is the concentration of diphacinone residues (ppm = mg diphacinone/kg gastropod), and *PT* is the fraction of gastropods consumed in treated area. Because diphacinone dietary toxicity data are available for northern bobwhite quail and mallard (Table 3), these species can be used to estimate secondary risks to birds consuming gastropods in diphacinone-baited areas in Hawaii. For a worst-case scenario, we assumed that *PD* and *PT* were equal to one; the diet of these birds consisted entirely of gastropods containing 4.93 ppm diphacinone (the upper 95% confidence limit for laboratory-exposed gastropod diphacinone residues). For adult quail and mallard, the diphacinone LC50 values are >1,250 and 2,265 ppm, respectively (Table 3). Dividing the dietary diphacinone concentration (4.93 ppm) by the LC50 values yielded a risk quotient of <0.0004 for adult quail and 0.002 for adult mallards. For juvenile quail and mallards, the LC50 values are >5,000 and 906 ppm, which yield risk quotients of <0.001 and 0.005, respectively. Because all these risk quotients are less than the 0.5 level of concern, the risks of diphacinone-induced mortality to nonthreatened or nonendangered birds consuming diphacinone-containing gastropods appear to be acceptable.

In addressing such risks on Hawaii, the Poouli (*Melamprosops phaesoma*), an endangered native Hawaiian bird, must be considered. The Poouli likely represents the honeycreeper species of greatest concern; of all endangered honeycreeper species, Poouli populations are the smallest. Insects and snails constitute a significant portion of the Poouli diet [27]. Because no diphacinone toxicity data exist for the Poouli, toxicity data from surrogate species must be employed. For this risk assessment, it was assumed that the diphacinone LD50 for the adult Poouli is less than 2,265 ppm, which is equivalent to that of the most-sensitive species (mallard). Examination of the range of reported mammalian toxicity values for diphacinone suggests an interspecies range for acute toxicity between one to two orders of magnitude (50-fold; Table 4). For birds, there are insufficient data to estimate interspecies sensitivity to diphacinone; toxicity values are documented for only mallard and northern bobwhite quail. However, such data exist for the rodenticide brodifacoum, an anticoagulant with the same mode of action as diphacinone [37]. The range of mammalian interspecies sensitivity to brodifacoum (86-fold) is between one and two orders of magnitude, which is similar to the 50-fold range for diphacinone LD50 values. The range of avian interspecies sensitivity to brodifacoum is 38.5-fold (Table 5); given the similarity in the range of mammalian interspecies sensitivities for diphacinone and brodifacoum, we assumed that the range for avian sensitivity to brodifacoum also was applicable to diphacinone. To extrapolate quail LD50 values to Poouli, each mallard LD50 value was divided by 38.5. This is consistent with the conservative assumption that the Poouli are more sensitive to diphacinone than are mallard.

Table 2. Residues of diphacinone in field-exposed snail and slug tissues

Species	Range of residue concn. (ppm)[a]	Mean residue concn. (ppm)[b]	95% Percentile residue concn. (ppm)	*t* Tests (LSD)[c]
Snails				
Oxychilus spp. $n = 3$	0.59–0.79	0.69	0.78	A
Slugs				
Limax maximus $n = 3$	0.60–0.61	0.61	0.61	A
Deroceral leave $n = 3$	0.21–0.25	0.23	0.25	B
Unknown species $n = 2$	0.56–0.68	0.62	0.67	A

[a] ppm = Parts per million.
[b] To calculate the mean residue for samples reported as <MLOD (method limit of detection), the MLOD was used as the value for these samples.
[c] LSD = Last significant difference; means with the same letter are not significantly different ($p = 0.005$).

Table 3. Deterministic avian secondary risk assessments; LC50 = Median lethal concentration

Species	Diphacinone residue concn. (ppm)[a]	LC50 (ppm)	Risk quotient	Level of concern
Northern Bobwhite Quail	4.93	>1,250[b]	<0.0004[b]	0.5
		>5,000[c,d,e]	<0.001[c]	
Mallard	4.93	2,265[b]	0.002[b]	0.5
		906[c,d,f]	0.005[c]	
Po'ouli	4.93	58.8[b]	0.08[b]	0.1
		23.5[c]	0.21[c]	

[a] Upper 95th percentile for diphacinone residue concentrations in *Deroceral* (laboratory-feeding study).
[b] Adult.
[c] Juvenile.
[d] U.S. Environmental Protection Agency [5].
[e] Long et al. [33].
[f] Long et al. [30].

Anticoagulant acute (LD50) and dietary (LC50) toxicity values for identical wildlife species are available only for the anticoagulant diphacinone and the species bobwhite quail and mallard. The range of LD50 values for these two species is 1.9, which is nearly identical to the 2.3-fold range of LC50 values (Table 5). Based on the similarity of LC50 and LD50 ranges, we also applied the 38.5 interspecies correction factor to the extrapolation of quail LC50 values to Poouli.

Because risk quotients for mallard and quail were less than the 0.5 level of concern, the deterministic approach indicated that broadcast distribution of diphacinone baits on Hawaii would be accompanied by an acceptable level of risk for nonthreatened avian species such as mallard and quail (Table 3). However, the juvenile Poouli risk quotient of 0.21 exceeds the 0.1 level of concern for threatened and endangered species. This suggests that a more detailed, probabilistic-based risk assessment was warranted to better estimate nontarget secondary risks to the Poouli.

Probabilistic risk assessment. The probabilistic-based 1-d exposure model predicted very low probabilities of mortality for exposed Poouli. Predicted levels of mortality were 0.03 (95% CI = 3.9×10^{-5} to 0.18) and 0.57% (95% CI = 2.6×10^{-4} to 4.28) for adult and juvenile populations, respectively (Fig. 5). Additionally, examination of percentile rankings for probability of mortality (data not shown) indicates that approximately 30% of adults and 80% of juveniles have greater than 0.01% (i.e., one in ten thousand) probability of mortality. Examination of the range of predicted mortalities (data not shown) indicate that the greatest probability of mortality for an individual Poouli is about 5.5% for adults and 28% for juveniles (i.e., no bird would have greater than 28% probability of dying due to diphacinone exposure).

For multiple-day exposures, each bird's exposure varied from day to day because different values for the quantity of invertebrates consumed and the diphacinone concentrations of the invertebrates were selected from the appropriate distributions. However, the same distributions were sampled each day because the residue analyses of the laboratory-exposed invertebrates indicated that the intraspecies diphacinone concentrations were not significantly different during the 7-d post-exposure period. Because diphacinone residues are persistent in invertebrates, it is probable that birds could consume diphacinone-containing invertebrates on multiple days. Given the persistence of rodenticides in animals, it is likely that diphacinone doses consumed on consecutive days would be at least partially cumulative. As such, it is logical that the 5-d exposure model predicted higher probabilities of mortality for Poouli than did the single-day exposure model. The mean mortality for exposed populations of Poouli were 3.2% (95% CI = 0.24–11.9) for adults and 7.7% (95% CI = 0.62–22.6) for juveniles. For this 5-d exposure scenario, the model predicted that 99.4% of adults and 100% of juvenile Poouli have greater

Table 4. Mammalian anticoagulant median lethal dose values (mg/kg)

Rodenticide	Rabbit	Dog	Cat	Pig	Range
Brodifacoum	0.29[a]	3.56[b]	25[a]	0.5[a]	25/0.29 = 86
Diphacinone	35[c]	3[d]	14.7[c]	150[e]	150/3 = 50

[a] U.S. Environmental Protection Agency [5].
[b] Godfrey [41].
[c] Interagency Program on Chemical Safety [37].
[d] Mount and Feldman [42].
[e] Kosmin and Barlow [43].

Table 5. Avian anticoagulant rodenticide toxicity values; LD50 = median lethal dose; LC50 = median lethal concentration

Species	LD50 (mg/kg)		LC50 (ppm)
	Brodifacoum	Diphacinone	Diphacinone
Northern bobwhite		1,630[a]	388[b]
Mallard	0.26[c]	3,158[c]	906[d]
Canada goose	<0.75[e]		
Black-backed gull	<0.75[e]		
Purple gallinule	0.95[e]		
California quail	3.3[e]		
Mallard	4.6[e]		
Black-billed gull	<5[e]		
Ring-necked pheasant	10[e]		
Australian harrier	10[e]		
Blackbird	>3[e]		
Hedge sparrow	>3[e]		
House sparrow	>6[e]		
Pukeko	0.95[e]		
Wax eye	>6[e]		
Range	38.5	1.9	2.3

[a] Campbell et al. [31].
[b] Shirazi et al. [25].
[c] U.S. Environmental Protection Agency [5].
[d] Long et al. [33].
[e] Godfrey [41].

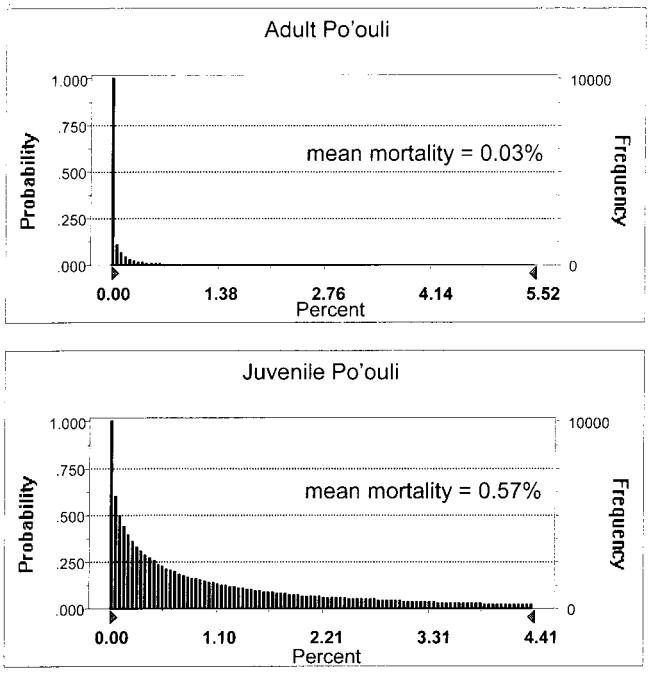

Fig. 5. Probability of mortality for adult and juvenile Poouli, single-day exposure.

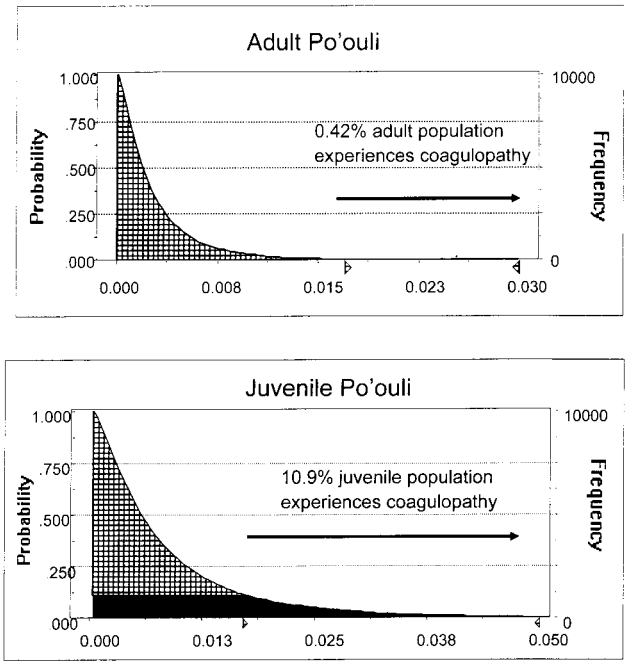

Fig. 6. Probability of coagulopathy in adult and juvenile Poouli based on 14-d exposure. Probability versus risk quotients (dose/LD50). LD50 = median lethal concentration.

than 0.01% probability of mortality. Examination of the range of predicted mortalities (data not shown) indicates that the maximum probability of mortality for an individual Poouli is about 30.4% for adults and 46.6% for juveniles.

Anticoagulant baiting programs with brodifacoum were associated with 20 to 100% reductions in avian populations [14,15]. The upper 95% confidence levels of mortality predicted by this model are less than 23%. These lower levels of mortality predicted for the broadcast application of diphacinone baits is consistent with the toxicity data presented in Table 5; brodifacoum is 3 to 4 orders of magnitude more toxic to birds than is diphacinone.

Evaluation of potential undesirable effects to nontarget wildlife, especially threatened or endangered species, should not be limited to mortality because nonlethal effects also could affect the survival of exposed individuals. For anticoagulant rodenticides, such effects include delayed prothrombin clotting times, which have been observed in diphacinone-exposed birds [21]. Prolonged prothrombin clotting times could result in a compromised ability to survive insults leading to external and/or internal bleeding. Unfortunately, prediction of the magnitude of delayed clotting times in Poouli is not possible because dose versus prothrombin clotting time data are not available for diphacinone-exposed birds. Given this paucity of data, an estimated no-adverse-effect-level risk quotient was generated based on the observed no-coagulopathy level for diphacinone-exposed rats [34]. In that study, the coagulopathy-no-effect level was 0.04 mg/kg body weight. The observed LD50 for diphacinone was 2.3 mg/kg body weight. Division of the no-effect level by the LD50 yielded a no-effect risk quotient of 0.017. Because levels of concern for risk quotients are applied routinely to a wide variety of species, we assumed that exposures associated with risk quotients less than 0.017 presented no detectable risks of coagulopathy [38]. Using the 14-d exposure probabilistic model, a distribution of risk quotients was generated for quail and Poouli. For both adult and juvenile quail, all subacute risk quotients for quail were less than the 0.017 level for concern. The predicted levels of measurable coagulopathy in exposed Poouli can be gleaned from the distributions for 10,000 adult and juvenile risk quotients (Fig. 6). The average risk quotient was <0.001 (95% CI = 0.00012–0.0093) for adults and 0.001 (95% CI = 0.00046–0.035) for juveniles. For Poouli consuming snails in a diphacinone-baited area, the model predicted that 4.0% of adults and 10.9% of juveniles would display measurable levels of coagulopathy. If exposure persists for more than 14 d, then these predicted levels of coagulopathy-positive Poouli likely would increase.

Delayed prothrombin clotting times were noted for eagles that had been exposed to 0.87 mg/kg body weight over 5 d [21]. Based on the average concentration of 1.77 ppm diphacinone, a Poouli would need to consume about 15.7 g of snails over 5 d to ingest this dose. This equates to consumption of less than 10% of the Poouli's body weight of snails each day. This seems quite plausible for a bird such as the Poouli, whose diet typically consists of about 60% snails [27]. This analysis reinforces the reasonableness of the model predictions; the risk of diphacinone-induced coagulopathy in Poouli may be significant.

Several assumptions included in the execution of this model may result in an overestimation of risk. For example, it was assumed that the all of the snails consumed by Poouli contained diphacinone residues. If a portion of snails are consumed outside of baited areas and do not contain diphacinone, the level of exposure would be less than predicted by the model. Also, only three field-collected snail samples were available for analysis, the distribution of snail diphacinone residues used for these predictions were based on residues observed in snails collected from the laboratory feeding study. In the few field-collected samples analyzed, the average residue concentration was less than half of that observed in the laboratory-collected samples. Although it would not be prudent to base toxicant exposure estimates for an endangered species on the results of three analyses, this observation suggests that it is very

Table 6. Assumption distribution parameters

Assumption	Units	Lower limit	Maximum (mean)	Upper limit	Standard deviation	Distribution	Pertinent model(s)[a]
Adult daily energy requirement	kJ/g bw	0.88	1.6	2.92	0.2	Lognormal	A, C
Juvenile daily energy requirement	kJ/g bw	1.38	2.9	5.76	0.2	Lognormal	A, C
Energy content of snails	kJ/g	1.39	1.15	1.61	0.05	Normal	A, C
Fraction of snails in diet	—	0	0.61	1	0.1	Normal	A, D
Energy content of insects	kJ/g	2.1	5.7	10.3	—	Triangular	A, C
Fraction of insects in diet	—	0	0.39	1	0.1	Normal	A, D
Diphacinone content in lab-exposed snails	ppm	0.35	1.77	3.19	0.47	Normal	A, C, D
Diphacinone content in field-exposed snails	ppm	0.53	0.69	0.85	0.07	Normal	A, C, D
Interspecies LC50 or LD50 extrapolation[b]	—	1	—	38.5	—	Uniform	A, D
Log LD50 northern bobwhite quail	mg/kg bw	2.24	3.14	4.04	0.3	Normal	A, C
Slope—log dose vs probit mortality curve	—	1.56	1.59	1.62	0.01	Normal	A
Log LC50 mallard	ppm	2.29	3.03	3.77	0.25	Normal	D
Slope—log concn. vs probit mortality curve	—	1.06	1.08	1.09		Normal	D

[a] A = acute toxicity model (Fig. 1); C = 14-d coagulopathy model (Fig. 3); D = 5-d dietary model (Fig. 2).
[b] LC50 = median lethal concentration; LD50 = median lethal dose; bw = body weight.

possible that diphacinone residues in snails consumed under field conditions would be less than those residues observed in the laboratory study. Mortality predictions based on the distribution of diphacinone residues observed in the field-collected snails were about 50% less than those based on the residues in laboratory-collected snails (data not shown). Coagulopathy predictions based on field-collected residue data were about 80% less than those modeled with laboratory-collected residue data (Table 2). Also, the use of allometric equations rather than species-specific energy utilization data for estimating Poouli energy requirements is another potential source of uncertainty that may impact model predictions.

Sensitivity analysis

Sensitivity analysis indicates that the most-significant assumptions (Table 6) for the mortality predictions in both the single-day and 5-d models are the values selected from the LD50 and the interspecies LD50 extrapolation distributions, suggesting that careful consideration of these variables are essential in constructing a valid model. Significant, although less important, variables include the diphacinone residue concentrations in the snails and the fraction of snails in the Poouli diet.

Probabilistic risk assessment

Compared to the widely used deterministic approach, probabilistic risk assessments provided a higher degree of refinement for characterizing risk. Deterministic approaches use fixed values to estimate toxicity and exposure and generate a single measure of risk, such as a risk quotient. Uncertain and variable parameters are fixed to nearly worst-case estimates. As such, the deterministic approach is very conservative and tends to overestimate risk; when a deterministic risk assessment indicates an acceptable level of risk, no further risk characterization is warranted. However, when the deterministic assessment suggests an unacceptable level of risk, as was the case for Poouli that consume gastropods in diphacinone rodenticide–baited areas, a more refined probabilistic assessment is warranted [39,40].

Risk management

The mortality estimates provided by this model provide risk managers with valuable information for weighing the risks against the benefits of the proposed baiting program. These mortality estimates can be compared directly to estimates generated for the evaluation of alternative proposed baiting strategies, or the mortality estimates subsequently may be incorporated into population models to permit the estimation of long-term population effects associated with the proposed baiting programs. In any case, by generating mortality (or subacute effects) estimates, we strongly believe that the modeling approach presented here offers a significant improvement over the widely used risk-quotient versus level-of-concern approach for determining ecotoxicological risks to nontarget wildlife.

The proposed use of diphacinone rodenticides to control invasive rat species in Hawaii is associated with a combination of ecological benefits and risk to nontarget native species. To maximize the ratio of benefits to risks, baiting strategies that minimize risk to endangered species should be further considered. In the case of the Poouli, only several birds are known to exist in Hawaii. For this reason, a small degree of risk may be unacceptable. For proposed baited areas that encompass the range of the Poouli, it may be feasible to evaluate baiting with a rodenticide that may present a much lower risk of secondary hazards, such as zinc phosphide. In areas outside of the Poouli's range, this risk assessment indicates the benefits of reducing pest rodent populations via broadcast distribution of diphacinone baits are accompanied by acceptable levels of risk to nonthreatened or nonendangered nontarget species. In this scenario, a myriad of native species could reap the benefits associated with diphacinone baiting for the reduction of invasive rat populations without risk to the vulnerable Poouli.

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