3/20/12

Comparative Risk Assessment of the First Generation Anticoagulant Rodenticide Diphacinone to Raptors

Barnett A. Rattner, Rebecca S. Lazarus and **Karen M. Eisenreich**

U.S. Geological Survey, Patuxent Wildlife Research Center, Beltsville, Maryland

Katherine E. Horak, Steven F. Volker, Christopher M. Campton and **John D. Eisemann**

U.S. Department of Agriculture, Animal and Plant Inspection Service, National Wildlife Research Center, Fort Collins, Colorado

Carol U. Meteyer

U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin

John J. Johnston

U.S. Department of Agriculture, Food Safety and Inspection Service, Fort Collins, Colorado

To whom correspondence may be addressed: Barnett A. Rattner, Ph.D.

Patuxent Wildlife Research Center

U.S. Geological Survey

10300 Baltimore Avenue, Beltsville Laboratory

BARC East-Building 308

Beltsville, Maryland 20705

Telephone: 301-497-5671

FAX: 301-497-5624

Email: brattner@usgs.gov

Abstract: New regulatory restrictions have been placed on the use of some second-generation anticoagulant rodenticides in the United States, and in some situations this action may be offset by expanded use of first-generation compounds. We have recently conducted several studies with captive adult American kestrels (*Falco sparverius*) and Eastern screech-owls **(***Megascops asio*) examining the toxicity of diphacinone (DPN) using both acute oral and short-term dietary exposure regimens. Diphacinone evoked overt signs of intoxication and lethality in these raptors at exposure doses that were 20 to 30 times lower than reported for traditionally used wildlife test species (mallard, *Anas platyrhychos* and Northern bobwhite, *Colinus virginianus*). Sublethal exposure of kestrels and owls resulted in prolonged clotting time, reduced hematocrit, and/or gross and histological evidence of hemorrhage at daily doses as low as 0.16 mg DPN/kg body weight. Findings also demonstrate that DPN was far more potent in short-term 7-day dietary studies than in single-day acute oral exposure studies. Incorporating these kestrel and owl data into the deterministic risk assessment recently conducted by the U.S. Environmental Protection Agency, and also into a probabilistic risk assessment, indicated that the risks associated with DPN exposure for raptors are far greater than predicted in analyses that principally used data from mallards and bobwhite. These findings can assist natural resource managers in weighing the costs and benefits of anticoagulant rodenticide use in pest control and eradication programs.

Key Words: anticoagulant, birds, clotting time, diphacinone, hazard, non-target effects, risk assessment, secondary poisoning

INTRODUCTION

Anticoagulant rodenticides are used for the control of vertebrate pests in urban and suburban settings, agriculture and island restoration projects. The goals of rodenticide application range from simple control of pest species to outright pest eradication for ecosystem restoration and recovery of native wildlife populations. Despite widespread use, there is growing concern of the risk to non-target wildlife, including endangered species (Erikson and Urban 2004). To reduce exposure and mitigate this risk, the U.S. Environmental Protection Agency (U.S. EPA) placed some restrictions on the sale, distribution and packaging of the second generation anticoagulant rodenticides (SGARs; e.g., brodifacoum, difethialone, bromadiolone and difenacoum) in 2008 (U.S. EPA 2011a). This action may result in expanded use of first generation anticoagulant rodenticides (FGARs) that are considered to be less hazardous to non-target wildlife than SGARs (Erikson and Urban 2004, Lima and Salmon 2010, Baldwin and Salmon 2011).

Nonetheless, even FGARs (e.g., chlorophacinone, diphacinone, warfarin) have been implicated in non-target wildlife mortality events. In a recent U.S. EPA report (2011b), diphacinone (DPN) was identified as the probable to highly probable cause of death in 16 unintentional wildlife mortality events. Several of these incidents involved secondary exposure of raptors consuming DPN-poisoned prey (red-tailed hawk, *Buteo jamaicensis*; barred owl, *Strix varia*; snowy owl, *Nyctea scandiaca*; turkey vulture, *Cathartes aura*) (U.S. EPA 2011b). In addition, DPN has been detected in tissues, but not definitely linked to mortality, in other birds of prey (Cooper's hawk, *Accipiter cooperii*, Stone et al. 2003; barn owl, *Tyto alba*, Pitt et al. 2005).

The U.S. EPA convened a scientific advisory panel (November 29-December 1, 2011) to review their analysis (U.S. EPA 2011b) of the potential risks to wildlife of four rodenticides (brodifacoum, difethialone, warfarin and bromethalin) for which a notice of intent to cancel was issued. The analysis also examined several likely alternatives, including the FGARs DPN and chlorophacinone. The U.S. EPA utilized existing acute oral and 5-day dietary exposure data derived in Northern bobwhite (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*), and other toxicity and field data, to conduct the risk assessment. Since 2009, we have been examining DPN toxicity and kinetics in both American kestrels (*Falco sparverius*) and Eastern screech-owls (*Megascops asio*) as part of an effort to develop a pharmacodynamic model and more complete avian risk assessment (Rattner et al. 2010a, 2011, 2012). These two species have been used extensively as toxicological models for raptors (Bardo and Bird 2009, Wiemeyer 2010). Based upon clotting time, histopathological, physiological and behavioral responses, and lethality, our studies indicate that raptors are considerably more sensitive to DPN than the traditionally used bobwhite and mallard test species. Herein, we compare and contrast the predicted hazards of DPN using traditional avian test species to our recently generated data on raptors by various risk assessment methods.

METHODS

Toxicity studies in American kestrels and Eastern screech-owls

Toxicity testing procedures in kestrels and owls were approved by the Institutional Animal Care and Use Committees of the Patuxent Wildlife Research Center

and the National Wildlife Research Center, and have recently been described in detail (Rattner et al. 2010a, 2011, 2012). Briefly, the median lethal dose (LD_{50}), associated statistics, and the lowest-observed-adverse-effect-level (LOAEL) were estimated in kestrels dosed using gelatin capsules multiple times in a 24-hr period (cumulative doses ranging from 35.1-675 mg/kg) with technical grade DPN (2-(diphenylacetyl)indan-1,3-dione; CAS 82-66-6; analytically verified at 99.2%; Hacco, Inc. Randolph, WI) and then observed for 7 days. In a separate kestrel study examining clotting time and DPN half-life, a single 50 mg/kg dose was administered and birds were weighed, bled and sacrificed at 6, 24, 48, 96 and 168 hrs post-dose (Rattner et al. 2011). Using similar methods, we attempted to conduct an acute oral toxicity test in Eastern screech-owls, however, serious problems were encountered with DPN regurgitation. The lowest lethal dose (LLD) was derived from this acute owl study. Subsequently, a short-term dietary toxicity test was conducted in which owls were fed graded concentrations of DPN mixed into Nebraska Bird of Prey diet (analytically verified to contain 0, 2.15, 9.55 and 22.6 ppm) for 7 days. Measurement of food intake, histopathological and physiological responses, clotting time (prothrombin time and Russell's viper venom time) and survival were monitored in this study (Rattner et al. 2012). The LOAEL, LLD, and lethal dietary concentration at which 33% of the owls succumbed (LC_{33}) were derived from these observations.

Toxicity data in other avian species

Acute oral and short-term dietary toxicity data for bobwhite and mallards were obtained from original reports submitted to the U.S. EPA (Fink 1976, Campbell et al. 1991, Long et al. 1992a, 1992b) or derived from other scientific papers or regulatory agency reports

(Eisemann and Swift 2006, Rattner et al. 2010b, U.S. EPA 2011b). These data were inspected, and in some instances raw data in these reports were used to calculate toxicity metrics for the risk assessment.

Statistical and risk analyses

The relation between neat DPN dose or dietary concentration and lethality in bobwhite, mallards, kestrels and owls was estimated using probit analysis (SAS Institute, Carey, NC, version 9.2 T2M3). The LLD and the LOAEL that evoked sublethal histopathological lesions or anemia (hematocrit < 30) were identified by simple inspection of the data. Continuously distributed variables (e.g., clotting time, hematocrit) were tested for homogeneity of variance (F_{max} test) and normality (Shapiro-Wilk test, normal probability plot and descriptive statistics) and then compared by analysis of variance and the Tukey's HSD test.

We used the standard guidelines employed by the U.S. EPA in their deterministic risk assessment of rodenticides (1998, 2011b), applying our data generated in kestrels and owls. The ratio of the dose or concentration to an endpoint was used to derive a risk quotient (RQ = exposure/toxicity). The RQ was then compared to a Level of Concern (LOC) for a non-target organism, with a value exceeding 0.5 indicating that the compound and associated use pattern presents an acute risk for non-listed species, and a value exceeding 0.1 indicating that endangered species may be potentially affected by use.

Probabilistic methods were also used in the present risk assessment. An exposure model was developed (Crystal Ball Software, Oracle Inc., Redwood City, CA) and used

to estimate the quantity of rodent liver consumption that would be required to exceed various toxicological endpoints in raptors (Johnston et al. 2005). The distributions of bodyweights and liver DPN concentrations were included in order to generate consumption estimate distributions and their associated probabilities. In addition, the DPN Benchmark Dose (estimate at which 10% of the test population exhibits a change in a specific endpoint; BMD_{10}) at which hematocrit was markedly depressed (value < 30 compared to 46.8 in controls) in owls was calculated using eight different models (gamma, multi-stage, Weibull, quantal-linear, logistic, log-logistic, probit and log-probit) for dichotomous data (Benchmark Dose software, BMDS Version 2.2; U.S. EPA 2011c). Models were evaluated based on the Akaike's Information Criterion.

RESULTS AND DISCUSSION

Acute oral toxicity of DPN to non-target avian species

For the adult mallard, the LD_{50} (95% confidence interval) was reported to be 3,158 mg/kg (1,605-6,211 mg/kg), and the LLD was 1000 mg/kg (Fink 1976). Another acute oral toxicity study conducted in adult bobwhite yielded questionable results as doses were separated by a factor of 5, no slope could be estimated, and 95% confidence intervals ranged from 0 to infinity (Campbell et al. 1991). The LD_{50} derived from this study has been reported as "400 mg/kg < LD_{50} < 2000 mg/kg" (Erikson and Urban 2004), although these data were re-evaluated, and a binomial model provided an adequate fit yielding an LD_{50} of 1,630 mg/kg (U.S. EPA 2011b). A recent study in bobwhite derived a more reliable LD_{50} (95% confidence interval) that was estimated to be 2,014 mg/kg

(1,620 - 2,475 mg/kg), and the LLD was 917 mg/kg (Rattner et al. 2010b). An acute oral toxicity study in American kestrels yielded an LD_{50} of 96.8 mg/kg with a 95% confidence interval of 37.9 to 219 mg/kg, and the LLD was 79 mg/kg (Rattner et al. 2011). The kestrel median lethal dose was over 15 times less than mallard and bobwhite values used by the U.S. EPA in their risk assessment (U.S. EPA 2011b). In related acute exposure studies in kestrels (Rattner et al. 2010b, 2011), adverse effects on clotting time were found at 50 mg/kg and histopathological evidence of hemorrhage was apparent at 35.1 mg/kg (LOAEL). The acute oral DPN toxicity trial in Eastern screech-owls failed to yield a dose-response relation, presumably because of regurgitation of the administered DPN (Rattner et al. 2012). Quantification of regurgitated DPN to adjust administered dose to retained dose still failed to produce a dose-response curve. This acute oral dosing trial did yield a LLD of 171.2 mg/kg, and signs of overt intoxication (subdued behavior, bruise on featherless tract, blood on vent and in droppings), coagulopathy and histopathological lesions were apparent at retained doses as low as 130 mg/kg.

The U.S. EPA examined the avian hazard associated with consumption of bait containing 50 ppm DPN for one day (U.S. EPA 2011b). In their deterministic risk assessment, the U.S. EPA selected the lowest avian LD_{50} value, which happened to be derived using bobwhite, and then adjusted this value using a body weight scaling factor (Mineau et al. 1996) for a generic 100 g bird (Table 1). We used a similar approach to scale an American kestrel LD_{50} value. For a diet containing 50 ppm DPN (concentration used in Ramik® Green bait, Hacco, Inc.), food intake rate for a 100 g generic bird and a kestrel (U.S. EPA 1993) was used to calculate a single-day DPN dose. The RQ (i.e., DPN dose/LD_{50}) was 21 times greater for the kestrel than for bobwhite. Although both

RQs were below the LOC, the value for kestrels (0.0939) approached the threshold (i.e., 0.1) for endangered birds. However, the actual risk is considerably lower as it is highly unlikely that a raptor would encounter DPN at a concentration approaching 50 ppm. Following broadcast application of DPN (0.005% in grain-based pellets) in field trials Hawaii, the extreme value in liver tissue of black rats (*Rattus rattus*) was 12 mg/kg and the extreme value found in house mice (*Mus musculus*) was 3.8 mg/kg (E.B. Spurr, U.S. Geological Survey, Pacific Island Ecosystem Research Center, Honolulu, HI, unpublished data). Furthermore, raptors would not directly consume the 50 ppm DPN bait pellets.

The U.S. EPA examined the avian hazard associated with consumption of mice with varying DPN body burdens for one day (U.S. EPA 2011b). In this assessment the DPN body burden (including half-life elimination) of a house mouse consuming 50 ppm DPN for 1, 3 and 6 days was estimated, and RQs were calculated using food intake rates for a generic bird feeding upon the exposed mice for a 24-hr period (Table 2). The RQ was well-below the LOC for generic birds. However, when re-analyzed using data from the body weight adjusted American kestrel LD_{50}, the LOC for an endangered bird was exceeded in several exposure scenarios.

In a previous report (Rattner et al. 2011), black rat liver DPN residue data (extreme value = 12 mg/kg) and American kestrel toxicity data were used to evaluate the risk to the endangered Hawaiian hawks (*Buteo solitarius*). Using a deterministic approach, exceeding the LD_{50} or even exceeding the LOAEL for histopathological lesions would require a 450 g Hawaiian hawk to consume over 1300 g of rat liver in a 24-hr period. This is an unrealistic scenario. However, applying the kestrel dose-response

curve for lethality in the probabilistic-based one-day exposure model (Johnston et al. 2005), it was predicted that 50% of male endangered Hawaiian hawks would have a 1% probability of mortality if they consumed only 3.5 g of liver from DPN-poisoned rats (Rattner et al. 2011).

The hazard of DPN in an acute exposure scenario using data derived from American kestrels is far greater than predicted from studies using the traditional bobwhite and mallard test species. The hazard may warrant more stringent review in a field setting. However, both laboratory and field studies indicate that FGARs generally require multiple feedings over several days to evoke mortality in target species (Ashton et al. 1986, Jackson and Ashton 1992). That is, repeated multi-day exposure greatly enhances FGAR toxicity. A recent critique on the use of the standardized acute oral avian toxicity test for generating FGAR toxicity and kinetic data suggests that this exposure regimen underestimates the hazard posed by environmentally relevant multiple-feeding scenarios, and can even mislead ecological risk assessment and forensic investigations (Vyas and Rattner 2012). Accordingly, the hazard of DPN in a multi-day exposure regimen was also investigated.

Short-term dietary toxicity of DPN to non-target avian species

In a 5-day dietary exposure trial using 10-day old mallard ducklings, the LC_{50} was reported to be 906 ppm DPN with a wide 95% confidence interval (187 - 35,107 ppm) (Long et al. 1992a). Inspection of the data in this report revealed that a duckling receiving a dietary concentration of 8 ppm succumbed on day 3 of the 5 day exposure period. Based upon its body weight (~259 g) and reported food consumption (75

g/bird/day), it is estimated that this duckling had ingested about 2.32 mg DPN/kg body weight/day (cumulative dose = 6.96 mg/kg over 3 days), which we identified as the LLD. It is noteworthy that the cumulative ingested dose for this duckling (i.e., 6.96 mg/kg) was over 140 times less than the 24-hr single oral dose evoking mortality (i.e., 1,000 mg/kg) in adult mallards (Fink 1976). It is not clear if this difference is due to greater sensitivity of ducklings compared to adults, or whether it is due to a dietary multi-day exposure versus the acute single-day exposure regimen. In a 5-day exposure trial using 10-day old bobwhite, the LC_{50} was reported as > 5,000 ppm (Long et al. 1992b).

In a 7-day feeding trial with adult Eastern screech-owls, 2 of 6 birds succumbed at a dietary concentration of 22.6 ppm. In this study, daily food consumption for each bird was determined, and the LLD was estimated to be only 0.82 mg/kg/day (cumulative dose = 5.75 mg/kg over 7 days). It is noteworthy that the cumulative 7-day ingested dose for this adult owl (i.e., 5.75 mg/kg) was over 150 times less than the LLD observed in adult mallards or bobwhite orally administered DPN in a 24-hr period (Fink 1976, Rattner et al. 2010). Furthermore, the 7-day dietary LLD for the most sensitive owl (i.e., 5.75 mg/kg) was nearly 30 times lower than the LLD (171.2 mg/kg) derived from adult owls in an acute oral dosing study, more definitively demonstrating the increased potency of FGARs when administered in a continuous multi-day low-level exposure scenario. Sublethal responses in owls occurred at DPN doses that were more than an order of magnitude lower than the LLD. Reduced hematocrit (< 30) for the most sensitive owl (LOAEL) was observed at a dietary dose of 0.36 mg/kg/day for 7 days, and for the entire data set the BMD_{10} for reduced hematocrit was 0.17 mg/kg/day for 7 days. All owls ingesting DPN exhibited prolonged clotting time when compared to controls, with a LOAEL for the 2.15

ppm group being 0.24 mg/kg/day, and for the most sensitive individual occurring at 0.16 mg/kg/day. This LOAEL for prolonged clotting time in screech-owls is quite similar to that reported in golden eagles that were fed meat from DPN treated sheep (*Ovis aries*) (i.e., 0.11 mg/kg/day) (Savarie et al. 1979, Eisemann and Swift 2006).

In their recent deterministic risk assessment, the U.S. EPA examined the risk associated with dietary exposure to 50 ppm DPN for 5 days to a generic bird (U.S. EPA 2011b). Using the LC_{50} for mallard ducklings (906 ppm), the RQ (i.e., DPN dose/LC_{50}) was 0.06 and below the LOC (Table 3). In our Eastern screech-owl study, 2 of 6 birds succumbed at 22.6 ppm in a 7-day exposure trial, and this response was used to approximate an LC_{33} as an LC_{50} value is not available for raptors (Rattner et al. 2012). The available data for the mallard (Long et al. 1992a) was re-evaluated by probit analysis to obtain an LC_{33} (i.e., 133 ppm, 95% confidence interval of 10.6 - 860 mg/kg). Using this mallard LC_{33} and a diet containing 50 ppm DPN, the RQ exceeded the LOC for only endangered avian species. However, by substituting the Eastern screech-owl LC_{33}, the RQ exceeded the LOC for all avian species, suggesting that the hazard to raptors may be greater than predicted using data from mallard ducklings. It is important to note that using the LC_{33} will result in a greater likelihood of exceeding the LOC but a smaller segment of the population may be at risk.

The avian risk associated with short-term dietary exposure to mice with varying DPN burdens was also examined (U.S. EPA 2011b). Using the 5-day mallard LC_{50} and the quantity of DPN accumulated in a house mouse over 3-days, the RQ for a generic bird was 0.023, well-below the LOC. In their analysis, the U.S. EPA did not apply an adjustment for differences in food intake of various sized birds, which overestimates risk

and errs on the side of safety. Herein, we used the LC_{33} for the mallard duckling and for the Eastern screech-owl, and the expanded the calculation to account for differences in food intake among birds of various sizes and for varying DPN doses (quantities accumulated in mice for 1, 3 or 6 days) (Table 4). Using the LC_{33} derived in mallard ducklings, the RQ was low, except for the exposure scenario in which a generic bird exclusively consumed mice for 5 days that had ingested and accumulated DPN from a 50 ppm bait for 6 days. In this case, the LOC was exceeded for an endangered bird. However, using the LC_{33} derived from the Eastern screech-owl, the RQ exceeded the LOC for endangered birds in all 3 exposure scenarios, and the RQ exceeded the LOC for all birds at the extreme 6-day mouse DPN concentration.

We recently described a deterministic evaluation in which the LLD of DPN in Eastern screech-owls was used to predict the hazard to the endangered Hawaiian hawk consuming liver from DPN-poisoned black rats, and to the state endangered Hawaiian short-eared owl (*Asio flammeus sandwichensis*) consuming liver from DPN-poisoned house mice (Rattner et al. 2012). Using the extreme DPN concentrations found in rodent liver, a 450 g hawk and a 350 g short-eared owl would have to consume unrealistically large quantities of rodent liver (> 30 g and > 75 g, respectively) for 7-days to evoke mortality. However, using a probabilistic exposure model (Johnston et al. 2005), consumption of 9.3 g of liver from DPN-poisoned black rats by Hawaiian hawks for 7-days, and consumption of 12.7 g of live from DPN-poisoned house mice by Hawaiian short-eared owls for 7-days would likely evoke mortality in 1% of the exposed male population of these species (Figures 1 and 2). Sublethal effects, such as prolonged clotting time, were estimated to occur in 1% of the populations of exposed hawks and

owls consuming 2.73 g/day of rat liver and 3.72 g/day of mouse liver, respectively.

CONCLUSIONS

Rodenticides have become fundamental for the control of vertebrate pest species in urban, suburban and agricultural settings, and in remote island restoration projects. The hazards associated with exposure of non-target wildlife to SGARs, and even some FGARs, have come to light over the past decade, and additional regulatory actions (labeling restrictions) were initiated in 2008 to mitigate risk (U.S. EPA 2011a). From a non-target wildlife perspective, FGARs are generally accepted as being less persistent and safer alternatives than SGARs. Nonetheless, the FGARs may pose a hazard to non-target wildlife in some settings and use patterns. Empirical toxicological data from controlled laboratory studies have recently demonstrated that American kestrels and Eastern screech-owls are considerably more sensitive to the FGAR DPN than bobwhite and mallards (Rattner et al. 2010a, 2010b, 2011, 2012). Results of deterministic and probabilistic risk assessments described in the present paper indicate that the risk associated with DPN, and perhaps other FGARs, is considerably greater than predicted from studies in bobwhite and mallards.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. Nancy H. Golden and Nimish B. Vyas and for reviewing a draft of this manuscript. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

LITERATURE CITED

Ashton, A. D., W. B. Jackson, and H. Peters. 1986. Comparative evaluation of LD50 values for various anticoagulant rodenticides. Trop. Pest Manage. 32:187-97.

Baldwin, R. A., and T. P. Salmon. 2011. The facts about rodenticides. Wildl. Prof. 5:50-53.

Bardo, L., and D. M. Bird. 2009. The use of captive American kestrels (*Falco sparverius*) as wildlife models: A review. J Raptor Res. 43:345-364.

Campbell, S., K. A. Hoxter, and G. J. Smith. 1991. Diphacinone technical: An acute oral toxicity study with Northern bobwhite. Wildlife International, Easton, MD. Project No. 284-103. Submitted by Bell Laboratories, Inc, Madison WI. U.S. EPA MRID 422452-01.

Eisemann, J. D., and C. E. Swift. 2006. Ecological and human health hazards from broadcast application of 0.005% diphacinone rodenticide baits in native Hawaiian ecosystems. Proc. Vertebr. Pest Conf. 22:413-433.

Erickson, W., and D. Urban. 2004. Potential risks of nine rodenticides to birds and nontarget mammals: A comparative approach. Office of Prevention, Pesticides and Toxic Substances, U.S. EPA, Washington, DC. 230 pp.

Fink, R. 1976. Acute oral LD_{50} – mallard duck – technical diphacinone, final report. 9 pp. Study conducted by Truslow Farms, Inc. submitted by Velsicol Chemical Corp. (CDL:234422-K) to U.S. EPA on July 12, 1978.

Jackson, W. B., and A. D. Ashton. 1992. A review of available anticoagulants and their use in the United States. Proc. Vertebr. Pest Conf. 15:156-60.

Johnston, J. J., W.C. Pitt, R. T. Sugihara, J. D. Eisemann, T. M. Primus, M. J. Holmes, J. Crocker, and A. Hart. 2005. Probabilistic risk assessment for snails, slugs, and endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. Environ Toxicol Chem 24:1557-1567.

Lima, L. L., and T. P. Salmon. 2010. Assessing some potential environmental impacts from agricultural anticoagulant uses. Proc. Vertebr. Pest Conf. 24:199-203.

Long, R. D., J. Foster, K. A. Hoxter, G. J. Smith, and S. M. Campbell. 1992a. Diphacinone technical: A dietary LC50 study with the mallard. Project 284–102B. Conducted by Wildlife International, Ltd. Submitted by Bell Laboratories, Inc., Madison, WI. EPA MRID 424088–02.

Long, R. D., J. Foster, K. A. Hoxter, G. J. Smith, and S. M. Campbell. 1992b. Diphacinone technical: A dietary LC50 study with northern bobwhite. Project 284–101A. Conducted by Wildlife International, Ltd. Submitted by Bell Laboratories, Inc., Madison, WI. EPA MRID 424088–01.

Mineau, P., B. T. Collins, and A. Baril. 1996. On the use of scaling factors to improve interspecies extrapolation of acute toxicity in birds. Reg. Toxicol. Pharmacol. 24:24-29.

Pitt, W. C., J. D. Eisemann, C. E. Swift, R. T. Sugihara, B. Dengler-Germain, and L.

Driscoll. 2005. Diphacinone residues in free-ranging wild pigs following aerial broadcast of a rodenticide bait in a Hawaiian forest. Unpublished Report QA-1077, National Wildlife Research Center, Fort Collins, CO. 35 pp. \

Rattner, B. A., K. E. Horak, S. E. Warner, D. D. Day, and J. J. Johnston. 2010a. Comparative toxicity of diphacinone to northern bobwhite (*Colinus virginianus*) and American kestrels (*Falco sparverius*). Proc. Vertebr. Pest Conf. 24:146-152.

Rattner, B. A., K. E. Horak, S. E. Warner, and J. J. Johnston. 2010b. Acute toxicity of diphacinone in Northern bobwhite: effects on survival and blood clotting. Ecotoxicol. Environ. Saf. 73:1159-1164.

Rattner, B. A., K. E. Horak, S. E. Warner, D. D. Day, C.U. Meteyer, S. F. Volker, J. D. Eisemann, and J. J. Johnston. 2011. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environ. Toxicol. Chem. 30:1213-1222.

Rattner, B. A., K. E. Horak, R. S. Lazarus, K. M. Eisenreich, C. U. Meteyer, S. F. Volker, C. M. Campton, J. D. Eisemann, and J. J. Johnston. 2012. Assessment of toxicity and potential risk of the anticoagulant rodenticide diphacinone using eastern screech-owls (*Megascops asio*). Ecotoxicol**.** In Press. DOI10.1007/s10646-011-0844-5

Savarie, P. J., D. J. Hayes, R. T. McBride, and J. D. Roberts. 1979. Efficacy and safety of diphacinone as a predacide. Pp. 69-79 *in*: E. E. Kenaga (Ed.), Avian and Mammalian Wildlife Toxicology, ASTM STP 693, American Society for Testing Materials.

Stone, W. B., J. C. Okoniewski, and J. R. Stedelin. 2003. Anticoagulant rodenticides

and raptors: recent findings from New York, 1998-2001. Bull. Environ. Contam. Toxicol. 70:34-40.

U.S. EPA (United States Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook. Volume 1 of 2. U.S. Environmental Protection Agency. EPA/600R/93/187a.

U.S. EPA (United States Environmental Protection Agency). 1998. Reregistration eligibility decision (RED): Rodenticide cluster. EPA 738-R-98-007. Washington, DC. http://www.epa.gov/oppsrrd1/REDs/2100red.pdf

U.S. EPA (United States Environmental Protection Agency). 2011a. Final risk mitigation decision for ten rodenticides. http://www.epa.gov/pesticides/reregistration/rodenticides/finalriskdecision.htm

U.S. EPA (United States Environmental Protection Agency). 2011b. Risks of non-compliant rodenticides to nontarget wildlife. Background paper for scientific advisory panel on notice of intent to cancel non-RMD compliant rodenticide products. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP 2011-0718-0006

U.S. EPA (United States Environmental Protection Agency). 2011c. Benchmark Dose Software (BMDS) Version 2.2 R65 [Build: 04/13/2011]. National Center for Environmental Assessment. http://www.epa.gov/NCEA/bmds/index.html

Vyas, N. B., and B. A. Rattner. 2012. Critique on the use of the standardized avian acute oral toxicity test for first generation anticoagulant rodenticides. Human Ecol. Risk Assess. 17 pp. In Press

Wiemeyer, S. N. 2010. Use of captive Eastern screech-owls (*Megascops asio*) as a wildlife model. J. Raptor Res. 44:251-252

Headings for Tables

Table 1. Acute avian risk associated with single-day exposure to 50 ppm DPN

Table 2. Acute avian risk associated with single-day ingestion of mice with varying DPN body burdens

Table 3. Avian risk associated with dietary exposure to 50 ppm DPN for 5 to 7 days

Table 4. Avian risk associated with 5 to 7 days of dietary exposure to mice with varying DPN burdens

Figure Legends

Figure 1. Cumulative probability curve of exceeding the LLD by male Hawaiian hawks consuming liver from DPN-poisoned black rats for one-week. One percent of the population (o) would exceed the LLD by consuming 9.3 g/day and 10% of the population (- - -) would exceed the LLD by consuming 55.9 g/day.

Figure 2. Cumulative probability curve of exceeding the LLD by male Hawaiian short-eared owls consuming liver from DPN-poisoned mice for one-week. One percent of the population (o) would exceed the LLD by consuming 12.7 g/day and 10% of the population (- - -) would exceed the LLD by consuming 66.9 g/day.

Table 1. Acute avian risk associated with single-day exposure to 50 ppm DPN

Measurement	Generic Bird[a] (derived from Bobwhite)	Raptor (derived from Kestrel)
Weight (g)	100	100
Weight Adjusted LD_{50} (mg/kg)	1480	95.8
Food Intake (g/day)	13	18
DPN Intake (mg/kg body weight/day)	6.5	9
Risk Quotient (DPN Dose/LD_{50})	0.0044	0.0939
Level of Concern (0.5)	No	No
Level of Concern for Endangered Species (0.1)	No	Approaching 0.1

[a]U.S. EPA 2011b

Table 2. Acute avian risk associated with single-day ingestion of mice with varying DPN body burdens

Measurement	Generic Bird[a] (derived from Bobwhite)	Raptor (derived from Kestrel)
Weight (g)	100	100
Weight Adjusted LD_{50} (mg/kg)	1480	95.8
Food Intake (g dry weight/day)	13	18
DPN Intake for 1-day mouse(mg/kg body weight/day)	3.21	4.44
Risk Quotient (DPN Dose/LD_{50})	0.0022	0.0463
DPN Intake for 3-day mouse(mg/kg body weight/day)	8.54	11.82
Risk Quotient (DPN Dose/LD_{50})	0.0057	0.1234
DPN Intake for 6-day mouse(mg/kg body weight/day)	14.4	19.94
Risk Quotient (DPN Dose/LD_{50})	0.0097	0.2081
Level of Concern (0.5)	No	No
Level of Concern for Endangered Species (0.1)	No	Yes for 3-day and 6-day

[a]U.S. EPA 2011b

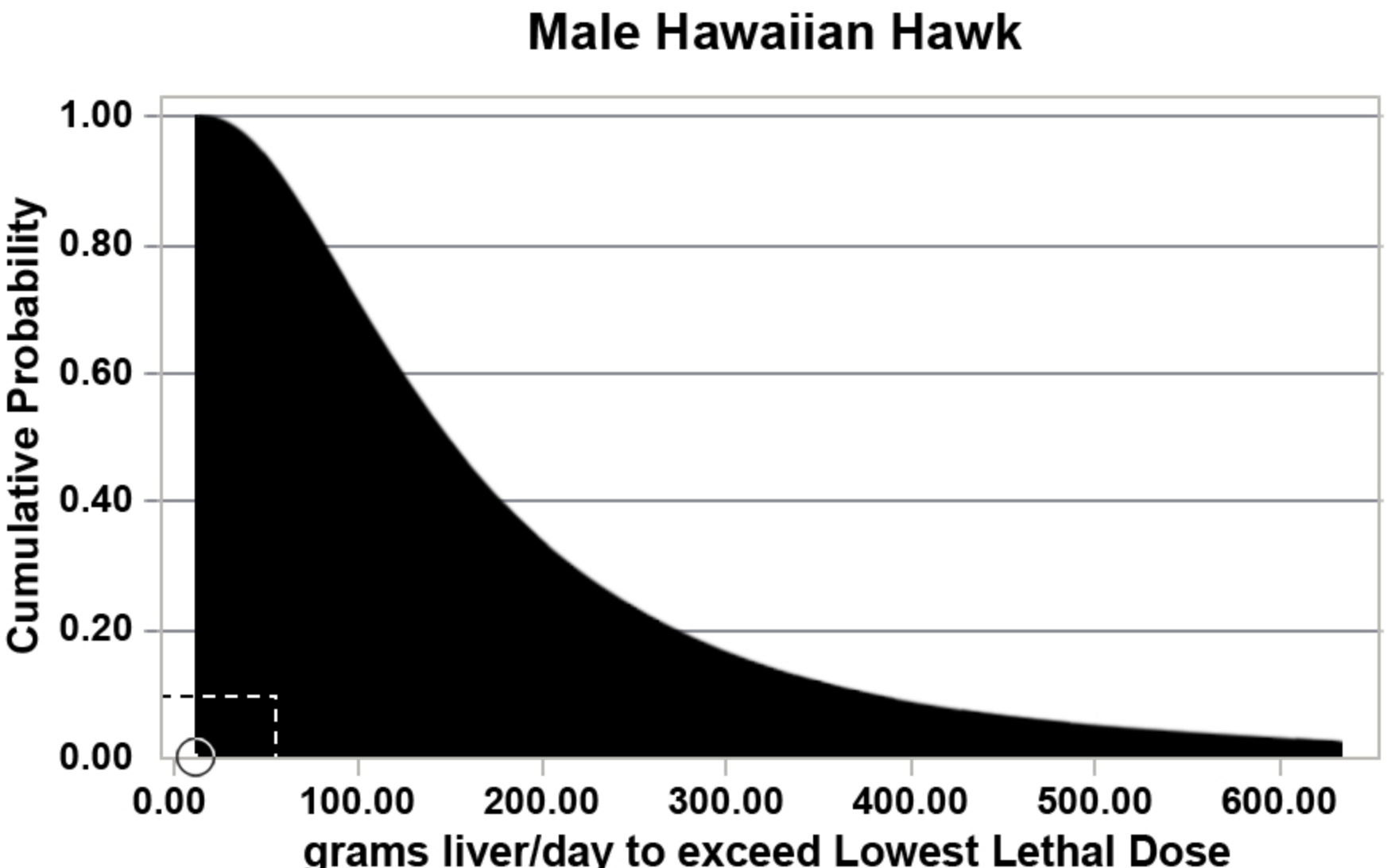
Table 3. Avian risk associated with dietary exposure to 50 ppm DPN for 5 to 7 days

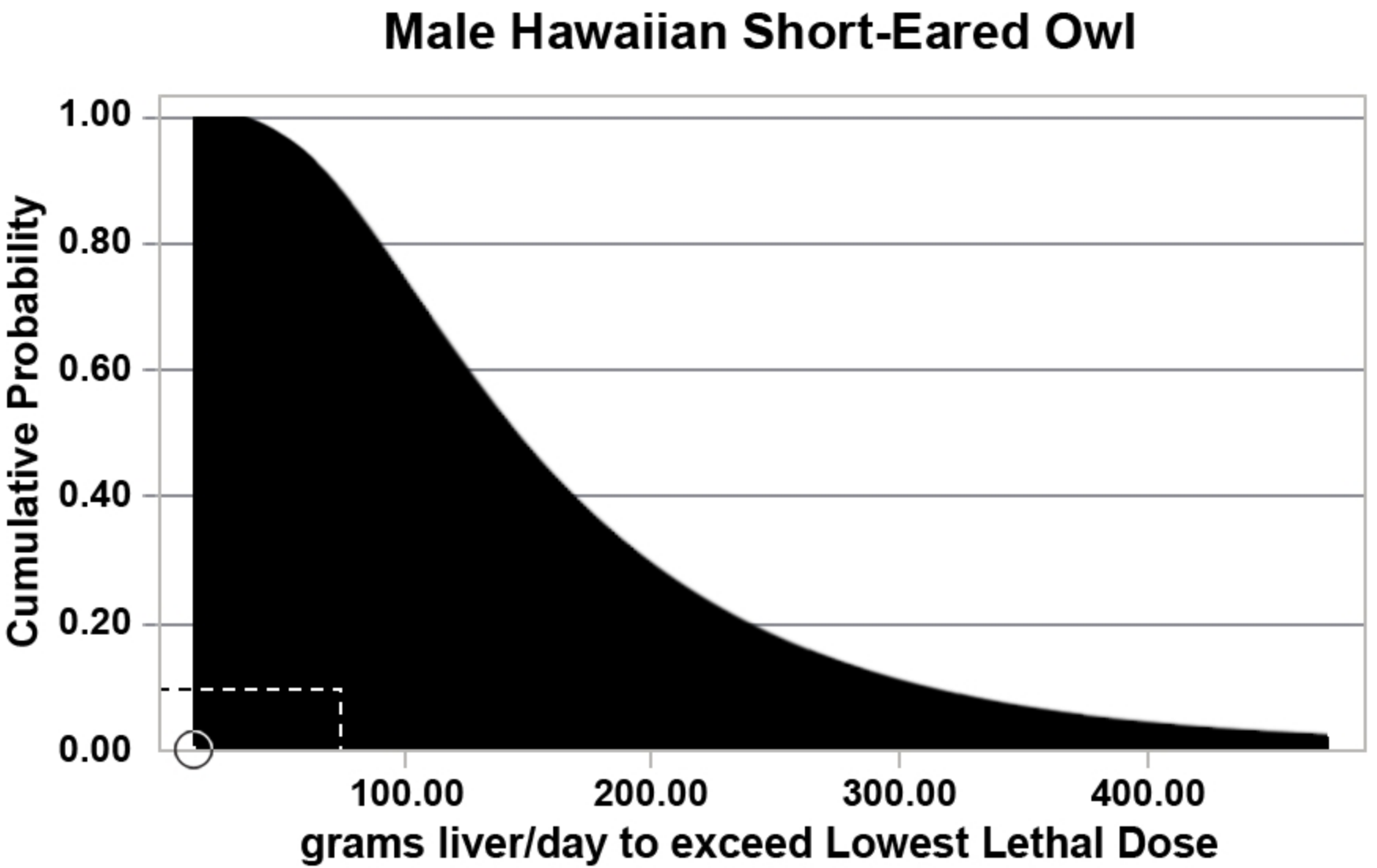
Measurement	Generic Bird[a] (derived from Mallard)	Raptor (derived from Screech-Owl)
Dietary Concentration (ppm or mg/kg)	50	50
LC_{50} (mg/kg)	906	Not Available
Risk Quotient (DPN Dietary Concentration/LC_{50})	0.06	—
Level of Concern (0.5)	No	—
Level of Concern for Endangered Species (0.1)	Yes	—
LC_{33} (mg/kg)	133	22.6
Risk Quotient (DPN Dietary Concentration/LC_{33})	0.3759	2.2124
Level of Concern (0.5)	No	Yes
Level of Concern for Endangered Species (0.1)	Yes	Yes

[a]U.S. EPA 2011b

Table 4. Avian risk associated with 5 to 7 days of dietary exposure to mice with varying DPN burdens

Measurement	Generic Bird (derived from Mallard)	Raptor (derived from Screech-Owl)
Weight (g)	100	100
Food Intake (g dry weight/day)	13	13
LC_{33} (mg/kg)	133	22.6
DPN Intake for 1-day mouse(mg/kg body weight/day)	3.21	3.21
Risk Quotient (DPN Dose/LC_{33})	0.0241	0.142
DPN Intake for 3-day mouse(mg/kg body weight/day)	8.54	8.54
Risk Quotient (DPN Dose/LC_{33})	0.0642	0.3778
DPN Intake for 6-day mouse(mg/kg body weight/day)	14.4	14.4
Risk Quotient (DPN Dose/LC_{33})	0.1082	0.6372
Level of Concern (0.5)	No	Yes
Level of Concern for Endangered Species (0.1)	Yes for 6-day	Yes


Male Hawaiian Hawk
Cumulative Probability
1.00
0.80
0.60
0.40
0.20
0.00
0.00
100.00
200.00
300.00
400.00
500.00
600.00
grams liver/day to exceed Lowest Lethal Dose


Male Hawaiian Short-Eared Owl
1.00
0.80
0.60
0.40
0.20
0.00
Cumulative Probability
100.00
200.00
300.00
400.00
grams liver/day to exceed Lowest Lethal Dose