2016

Retention time of chlorophacinone in black-tailed prairie dogs informs secondary hazards from a prairie dog rodenticide bait

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Research Article

Received: 12 March 2015 Revised: 5 May 2015 Accepted article published: 21 May 2015 Published online in Wiley Online Library: 18 June 2015

(wileyonlinelibrary.com) DOI 10.1002/ps.4045

Retention time of chlorophacinone in black-tailed prairie dogs informs secondary hazards from a prairie dog rodenticide bait

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Abstract

BACKGROUND: Secondary toxicity in mammals and birds that consume animals containing residues of anticoagulant rodenticides represents a persistent conflict between conservation, agriculture and environmental contamination. Chlorophacinone residues in black-tailed prairie dogs (*Cynomys ludovicianus*) represent a secondary exposure hazard to predatory and scavenging avian and mammalian species in the Central Plains of the United States, especially considering efforts to re-establish black-footed ferrets (*Mustela nigripes*). Rozol® Prairie Dog Bait (chlorophacinone 0.005%) is registered to control black-tailed prairie dogs in ten states throughout the midwestern and western United States.

RESULTS: We fed Rozol Prairie Dog Bait to captive black-tailed prairie dogs for 2 days and analyzed their livers and whole bodies (without livers) for chlorophacinone residue on days 3, 5, 7, 9, 11, 14, 18 and 27 post-exposure. We found the greatest levels of residues in livers ($\bar{x} = 5.499$ mg kg^{-1}) and whole bodies ($\bar{x} = 1.281$ mg kg^{-1}) on day 3. Residues in both tissues declined rapidly over time, with estimated half-lives of approximately 6 days post-exposure. However, a risk assessment of secondary toxicity to non-target mammals indicated acute risks for mammalian species up to 27 days post-exposure and negligible risks for birds.

CONCLUSION: The results suggest that the greatest risk of secondary toxicity occurs ≤14 days post-application of Rozol Prairie Dog Bait and declines thereafter. This corresponds to the time when chlorophacinone residues are high, and prairie dogs exhibit signs of intoxication and are perhaps most susceptible to predation and scavenging. These results confirm that Rozol Prairie Dog Bait should not be used in areas where black-footed ferrets or other sensitive species occur.

Keywords: anticoagulant; poisoning; rodenticide; rodent damage; secondary hazards; toxicant

1 INTRODUCTION

Black-tailed prairie dogs (*Cynomys ludovicianus*) are the most widely distributed species of prairie dogs in North America, inhabiting grasslands from Montana to New Mexico.[1] Lethal control of populations with rodenticides occurs because of conflicts that arise between the black-tailed prairie dogs and humans, such as property damage, consumption of range forage meant for livestock, threat of plague to humans and companion animals and social attitudes about prairie dogs.[2] Historically, management of the prairie dog population included poisoning (e.g. 2% zinc phosphide grain baits), fumigants, barriers and relocation.[3–5] Anticoagulant rodenticides are registered for use on prairie dogs and are commonly used to control populations of black-tailed prairie dogs.[6]

The anticoagulant rodenticide chlorophacinone was demonstrated to be an effective rodenticide for prairie dogs when tested in cage trials[7] and when placed in burrows during field trials In 2012, Rozol® Prairie Dog Bait (chlorophacinone 0.005%; Liphatech, Milwaukee, WI) was registered for use to control prairie dogs in ten midwestern and western Unites States (EPA registration number 7173-286). Unlike traditional toxicants for prairie dogs (e.g. zinc phosphide), anticoagulant rodenticides such as Rozol persist in tissues for days to weeks after consumption of the bait.[9] Fisher and Timm[7] demonstrated the potential for secondary hazards to carnivores (using domestic ferrets) from consumption of prairie dogs that were exposed to chlorophacinone.

Signs of intoxication from exposure to chlorophacinone typically take several days after ingestion to manifest, and it may take 7–20 days for mortality to occur after a single dose (Yoder C, unpublished). Therefore, after consuming a dose, some exposed prairie dogs are likely predated or scavenged by other species. Many mammalian and avian species utilize prairie dogs as a food source, which generates concern about the potential for secondary poisoning of these animals. Of particular concern, the critically endangered black-footed ferret (*Mustela nigripes*) relies primarily on prairie dogs as food. Other animals (i.e. mustelids, owls and raptors) have already been found with chlorophacinone residues in their livers and had attributed deaths,[10–14] suggesting that secondary poisoning is occurring.

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To assess accurately the potential risks of secondary poisoning, reliable information on the levels of chlorophacinone residues in prairie dog tissues after exposure to Rozol Prairie Dog Bait is needed. Therefore, our primary objective was to determine the levels of chlorophacinone residues in the livers and whole bodies of black-tailed prairie dogs following exposure to Rozol Prairie Dog Bait. We predicted a peak in chlorophacinone residues that would dissipate over time. Our secondary objective was to conduct a risk assessment for mammalian and avian species that may be secondarily exposed to chlorophacinone by consuming prairie dogs. Information from both objectives will assist safety evaluations of the current registration label for Rozol Prairie Dog Bait.

2 EXPERIMENTAL METHODS

We captured 50 black-tailed prairie dogs from Buckley Air Force Base in Denver, Colorado, during January 2010 using 76 × 18 × 18 cm live-cage traps (Tomahawk Live Traps, Hazelhurst, WI). These prairie dogs were considered to be nuisance animals and were scheduled to undergo trapping and euthanasia. We captured adult prairies dogs (i.e. females of ≥600 g and males of ≥700 g) for this study. Captured prairie dogs were weighed, dusted for fleas (Drione; Bayer, Leverkusen, Germany) and transported in individual traps to the National Wildlife Research Center (NWRC) in Fort Collins, Colorado (a drive lasting less than 2 h).

Upon arrival at the NWRC, prairie dogs were individually housed indoors in 61 × 46 × 30 cm cages that were equipped with 30 cm sections of PVC pipe to serve as hides. We set the light conditions to 12 h of light and 12 h of dark. Prairie dogs were maintained on mostly grass hay supplemented with apples and carrots throughout the study to mimic their natural diet[14,15] and levels of vitamin K1 (i.e. the antidote to anticoagulant rodenticides).[16,17] We provided water *ad libitum*. We quarantined and monitored the health of the prairie dogs for 2 weeks before the study was initiated. This study was approved by the NWRC Institutional Animal Care and Use Committee (QA-1682) on 18 November 2009.

2.1 Chlorophacinone residues following exposure to Rozol Prairie Dog Bait

We randomly divided the prairie dogs into a treatment group ($n = 39$) of 19 males and 20 females and a control group ($n = 11$) of five males and six females. We removed food from all cages 12 h prior to initiation of the study to ensure the animals were motivated to feed. For animals in the treatment group on day 1, we provided 53 g (i.e. one-quarter cup) of Rozol Prairie Dog Bait in ceramic bowls with no alternative food. This exposure amount follows the Rozol Prairie Dog Bait label for active burrow treatments, which is purposefully set to limit the time of exposure. We allowed 2 days for consumption of this bait. For three treatment animals (one male and two females), we provided 150 g of the bait to determine whether the prairie dogs would consume more bait *ad libitum*. After 2 days, we removed and weighed all of the bait that was not consumed in each cages. After removing the bait, we provided the aforementioned maintenance diet to the treatment animals for the remainder of the study. Control animals were provided only maintenance diet throughout the study.

We monitored the prairie dogs twice daily (i.e. morning and late afternoon) for signs of toxicity and mortality status throughout the study. Any animals observed to be experiencing severe signs of pain or distress, or of a moribund condition (e.g. substantial lethargy, unresponsive to probing, substantial bleeding), were humanely euthanized.[18] Otherwise, four randomly selected animals (i.e. two males and two females) from the treatment group were euthanized on predetermined days 3, 5, 7, 9, 11, 14, 18 and 27 post-exposure to the rodenticide. Any animals that died or were euthanized because of condition were associated with the closest predetermined day. Two animals from the control group were euthanized on days 3, 11 and 22 post-exposure, and the five remaining control animals were euthanized on day 27 post-exposure. All animals were euthanized in gas chambers by firstly anesthetizing with isoflourane gas and then euthanizing with carbon dioxide gas.

After death, we prepared two samples from each prairie dog for chemical analysis of chlorophacinone residues. Firstly, we removed and immediately froze the livers, because anticoagulant rodenticides readily accumulate in liver tissue.[19] Secondly, we discarded the pelt, head, paws and tails from the body and immediately froze the remaining body (hereafter termed whole-body samples). For the purposes of this study, whole-body samples did not include livers. The whole-body samples provide information on potential exposure when an entire prairie dog is consumed by a predator or scavenger, including the digestive tract. After day 27, each liver and whole-body sample was thawed and homogenized. Approximately 1.00 g samples of each tissue were analyzed using reverse-phase ion-pair chromatography,[20,21] validated under NWRC Analytical Chemistry Methods 143A and 142A respectively, to determine chlorophacinone concentration. Seven fortified control samples were assayed following each of these methods, and no chromatographic interferences were observed. Chemical analysis methods for liver samples were validated for tissues containing 0.40 and 4.0 mg kg^{-1} of chlorophacinone. The efficiency of recovery averaged 101% (SD = 7.5%) and 103% (SD = 3.9%), respectively, in livers. Methods for whole-body samples were validated for tissues containing 0.20 and 2.0 mg kg^{-1} of chlorophacinone. The efficiency of recovery averaged 107% (SD = 2.0%) and 100% (SD = 4.5%), respectively, for whole bodies. The method limit of detection was 0.053 mg kg^{-1} for the liver and 0.061 mg kg^{-1} for the whole-body samples. If residues were below the method limit of detection in the liver or whole-body tissues for prairie dogs that consumed chlorophacinone, we used the limit values of 0.053 and 0.061 mg kg^{-1}, respectively, as the residue levels.

2.2 Statistical analysis of residues

We used R v.3.1.1 software (R Development Core Team, Vienna, Austria) to conduct analysis of variance (ANOVA) tests for comparing the residue levels of chlorophacinone in tissues over time. To compare the amount of Rozol Prairie Dog Bait that was consumed between animals that were offered 53 and 150 g of bait, *t*-tests were used. A linear regression model was employed to examine whether the amount of bait consumed was influenced by the weight of prairie dogs. We examined whether the amount of Rozol Prairie Dog Bait consumed influenced the residue levels of chlorophacinone detected in the livers and whole bodies with linear mixed-effects models using package lme4 (v.1.1-5). Temporal variation was accounted for by specifying the sampling days post-exposure as random effects. We calculated *F*-statistics and *P*-values based on Satterthwaite approximation for denominator degrees of freedom using the lmerTest v.2.0-6 package.

We used linear mixed-effects models to examine for effects from sex on the residue levels of chlorophacinone in the livers and whole bodies. Similarly, we used these models to examine for effects from animals that succumbed to the rodenticide versus

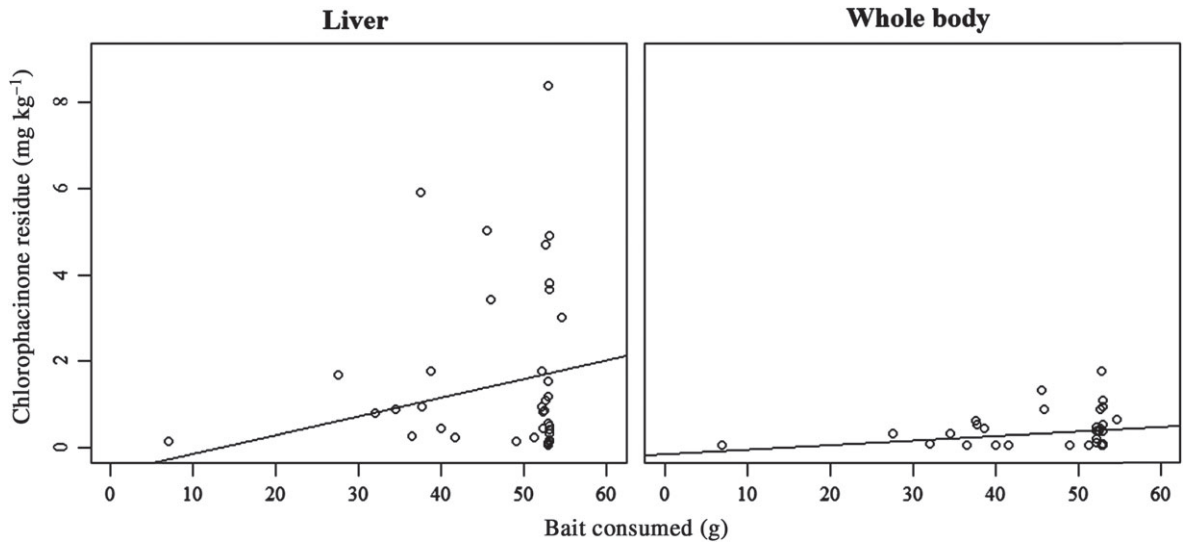

Figure 1. Estimated regression line showing the influence of Rozol Prairie Dog Bait consumed on the level of chlorophacinone residue detected using reverse-phase ion-pair chromatography in tissues of black-tailed prairie dogs (*Cynomys ludovicianus*).

animals that were euthanized during the same predetermined days on the residue levels of chlorophacinone. We considered *P*-values of <0.05 to indicate significant effects or differences in all tests. Finally, we conducted a locally weighted scatterplot smoothing (LOESS) regression to generate a decay curve and predict the temporal half-life of chlorophacinone residues in the tissues.

2.3 Risk assessment for secondary toxicity

We evaluated the risks of secondary toxicity to mammals and birds that may consume prairie dogs by calculating risk quotients (RQs).[13,19,22,23] The RQs were calculated by dividing animal exposure to chlorophacinone on consumption of whole-body carcasses (mg kg^{-1}) by the estimated weight-adjusted avian and mammalian median lethal acute oral doses (LD_{50}).[13] We calculated dry food consumption to assess risk for three standard weight classes of generic birds (50, 1000 and 5000 g) and mammals (50, 1000 and 3000 g).[13] Animal exposure to chlorophacinone was calculated by multiplying the dry food consumption by the average residual levels of chlorophacinone detected on days 3, 5, 7, 9, 11, 14, 18 and 27 post-exposure in the whole bodies of the prairie dogs. These averages represented conservative estimates of the residues that predators and scavengers may ingest, given that livers were not included in the whole-body samples. We estimated the weight-adjusted LD_{50} values following US EPA[13] and adjusting the LD_{50} values from the standard values reported for northern bobwhite quail (*Colinus virginianus*; LD_{50} = 258 mg kg^{-1}, average weight = 203 g; US EPA Master Record identification number 41513101) and for laboratory rats (*Rattus norvegicus*; LD_{50} = 6.26 mg kg^{-1}, average weight = 175 g; US EPA Master Record identification number 41875301).[13] We used a scaling factor of 1.15 for avian species[24] and body weight$^{3/4}$ for mammalian species.[25] We compared the RQ values to the acute level of concern described by the US EPA, where any values of ≥0.5 indicated a considerable acute risk of secondary poisoning to terrestrial animals.[26]

3 RESULTS

We did not detect differences in the amount of Rozol Prairie Dog Bait that was consumed between the animals offered 53 g of bait ($\bar{x}$ = 48.5, SD = 7.3) and animals offered 150 g ($\bar{x}$ = 33.0, SD = 24.1; t = 1.11, P = 0.3822), and therefore we pooled these animals for subsequent analyses. The average amount of bait consumed was 47.3 g (SD = 9.8). The prairie dogs consumed 7.0–54.6 g of Rozol Prairie Dog Bait in 2 days. The amount of bait consumed was not influenced by the weight of the prairie dogs ($F_{1,37}$ = 0.11, P = 0.7397). As prairie dogs consumed more bait, we detected increases in the residue levels in the liver ($F_{1,31}$ = 4.48, P = 0.0425) and whole-body tissues ($F_{1,31}$ = 5.98, P = 0.0202) (Fig. 1).

Residual chlorophacinone was below the method limit of detection for one liver and 14 whole-body tissues, and thus set to the limit values. Residue levels of chlorophacinone were higher in the liver than the whole-body tissues (t = 3.78, P = 0.0005). Residue levels declined significantly over time in livers ($F_{1,37}$ = 27.61, P < 0.0001) and in whole bodies ($F_{1,37}$ = 27.99, P < 0.0001) (Fig. 2). We did not detect an influence from sex on the residue levels in livers ($F_{1,31}$ = 0.81, P = 0.3751) or the whole bodies ($F_{1,31}$ = 0.83, P = 0.3703). The fitted decay curve indicated a temporal half-life of chlorophacinone residues at 5.9 days for the liver and 6.3 days for the whole body (Fig. 3).

The first clinical signs of intoxication by anticoagulant poisoning were observed on day 5 (i.e. lethargy) and more severely on day 8 (i.e. external bleeding or blood in feces) post-exposure. Of 12 animals that succumbed to the rodenticide bait, three died and nine were euthanized because they were considered to be moribund. The average days to death of these animals was 15.3 days (range 9–26 days). We did not detect any influence from animals that succumbed from ingestion of rodenticide bait compared with those that underwent scheduled euthanasia in residue levels in the livers ($F_{1,18}$ = 0.30, P = 0.5915) or the whole bodies ($F_{1,18}$ = 0.26, P = 0.6172). No chlorophacinone residues were detected in the control animals (i.e. all values were below the method limit of detection).

The risk assessment indicated declining risks of secondary toxicity to mammals through time (Fig. 4). Considerable acute risk was apparent for generic mammals during ≤5 days post-exposure, and the risk did not become negligible until approximately 14 days post-exposure. Risk to the generic avian species was negligible throughout.

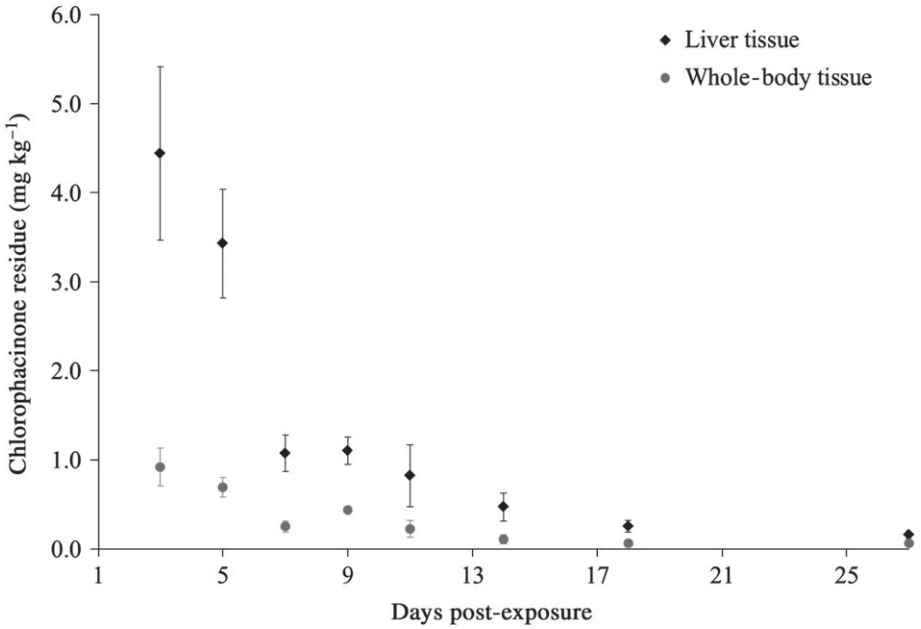

Figure 2. The mean with standard error bars for the level of chlorophacinone residue detected using reverse-phase ion-pair chromatography in tissues of black-tailed prairie dogs (*Cynomys ludovicianus*) post-exposure to Rozol Prairie Dog Bait.

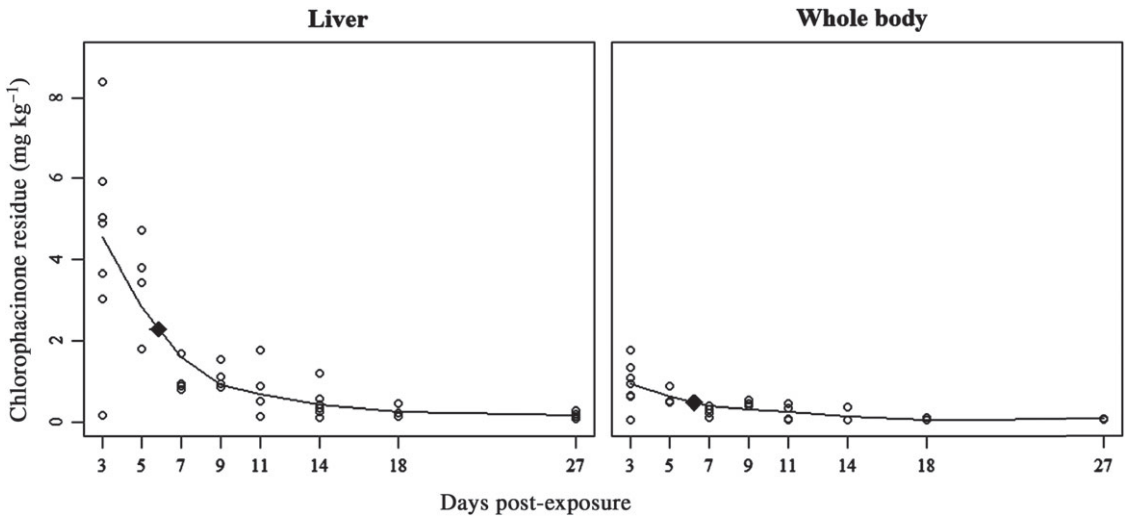

Figure 3. Decay curve generated with locally weighted scatterplot smoothing (LOESS) regression. Regression shows the fitted level of chlorophacinone residue detected using reverse-phase ion-pair chromatography in tissues of black-tailed prairie dogs (*Cynomys ludovicianus*) post-exposure to Rozol Prairie Dog Bait. Black dots represent the temporal half-life estimate for each tissue.

4 DISCUSSION

Results from this study suggest that chlorophacinone residues in prairie dogs from exposure to Rozol Prairie Dog Bait are initially high but decline faster than previously expected. The residues were highest during 3–6 days post-exposure, but declined sharply in subsequent days. A field study using Rozol bait found a similar sharp decline in residues in the livers of dead prairie dogs; however, residue analyses were not conducted until carcasses were first observed above ground ≥12 days post-exposure.[27] The residue levels on day 12 of this field study ($\bar{x} \sim 3.4$ mg kg^{-1}) closely matched the levels we detected on day 5 ($\bar{x} = 3.4$ mg kg^{-1}) post-exposure. These contradictory results have three possible explanations: (1) exposure times may be longer than 2 days in field applications; (2) consumption rates in field applications are higher than 53 g per 2 days; (3) collection of prairie dog carcasses above ground yields biased results because the majority of prairie dogs die underground and are undetected.[8] The field study used the same rate of Rozol application as mimicked in this experiment. Thus, if exposure time in field applications is longer than 2 days or consumption rates are higher than 53 g per animal, the results from this experiment represent conservative estimates of risk to non-target species. Our findings are confirmed by another laboratory study in which chlorophacinone bioaccumulated in the livers of laboratory rats for ≤4 days until equilibrium, indicating a rapid elimination of chlorophacinone.[28] The reported half-life for chlorophacinone in the livers of mice was much longer, reported as 35.4 days.[29]

The levels of chlorophacinone residues in the livers of black-tailed prairie dogs (0.06–8.4 mg kg^{-1}) were similar to those

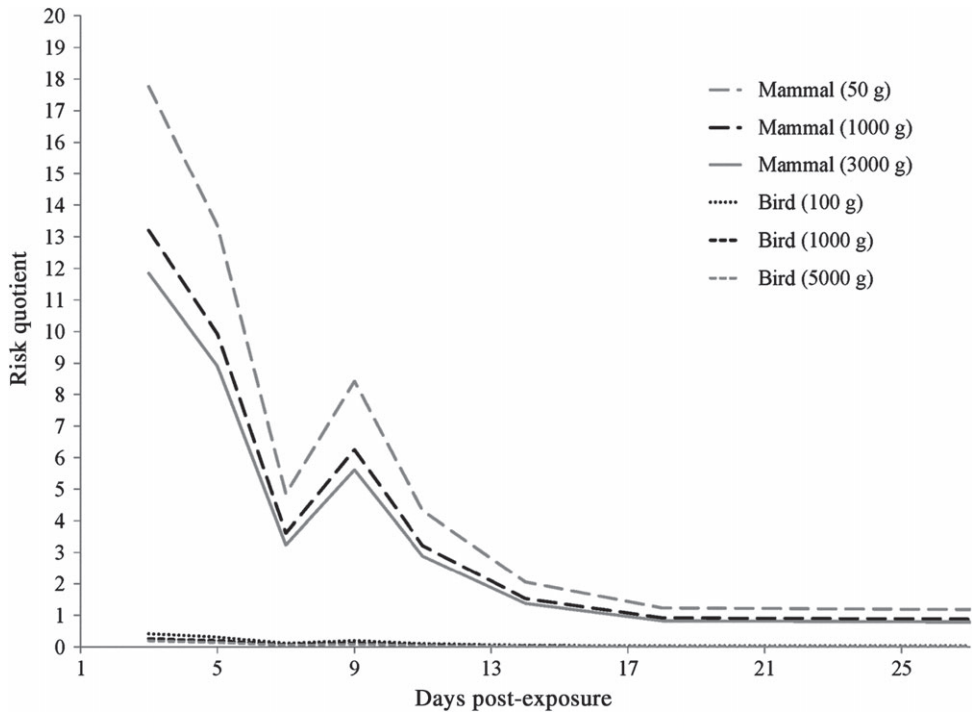

Figure 4. Risk quotients calculated using the average level of chlorophacinone residue detected using reverse-phase ion-pair chromatography in whole-body tissues of black-tailed prairie dogs (*Cynomys ludovicianus*) post-exposure to Rozol Prairie Dog Bait.

reported for prairie dogs in another study (0.44–7.56 mg kg^{-1}).[27] Compared with other species, the maximum residues from prairie dogs were between the maximum reported for laboratory rats (24.9 mg kg^{-1}) in a lab study[28] and those reported for Belding's ground squirrel (*Urocitellus beldingi*; 0.82 mg kg^{-1}) and *Microtus* spp. (4.1 mg kg^{-1}) during field studies in California.[23] Lower levels of chlorophacinone residues were also reported in the livers of voles (*Microtus arvalis*; 0.082–3.800 mg kg^{-1}).[30] Caution is warranted with these comparisons, given that time post-exposure generates variability in the residues of chlorophacinone in tissues.

The amount of bait consumed by prairie dogs influenced the level of chlorophacinone residues, as expected. The three prairie dogs we allowed to eat *ad libitum* consumed 7, 38 and 55 g of bait respectively. Although this is a small sample size with high variability, it provides little evidence that prairie dogs would consume exceedingly more than 53 g of bait in a 2 day period. Of the 36 animals offered 53 g of bait, nine animals consumed all the bait, 25 animals consumed ≥50 g and all animals consumed ≥28 g, which provides some indication that the label application rate is reasonable per active burrow. It would be possible for prairie dogs to consume ≥53 g in field applications, given that other prairies dogs will not consume it all.

The average number of days to death identified in this study match previous findings. In a previous LD_{50} study, most deaths occurred in 9–14 days, with a smaller peak in deaths in 17–20 days (Yoder C, unpublished report). We noticed that lethargy, the first observable symptom of anticoagulant poisoning, was apparent on day 5, when chlorophacinone residues in the tissues of prairie dogs were still quite high. This juxtaposition of symptomatic prairie dogs with unmetabolized concentrations of chlorophacinone is especially concerning for secondary toxicity, because lethargic prairie dogs may be more susceptible to predation. However, at the time of death ($\bar{x}$ = 15.3 days), tissue residues of chlorophacinone have declined substantially from day 3, and the risk of secondary poisoning is also reduced.

Using chlorophacinone to control prairie dogs has resulted in known poisonings of non-target species.[31] Our risk assessment indicated acute risks to mammalian predators and scavengers up to 27 days post-exposure. Similar to our findings, other studies found that mammalian animals were at acute risk to secondary toxicity, but avian species were not.[13,23] A report from the EPA[13] found slightly higher risks for mammals (RQ = 20.42) and birds (RQ = 0.32) that consumed house mice. Other studies have reported higher risk of secondary poisoning for avian species than found here. For example, risk of secondary toxicity was detected for barn owls (*Tyto alba*) from chlorophacinone in poisoned rats, although lower than the risk from bromadiolone.[32] Sublethal effects may be harder to identify but are equally important. Sublethal effects from chlorophacinone compromised the survival of free-ranging raptors, such as American kestrels (*Falco sparverius*),[33] and were also associated with reduced breeding performance in barn owls.[34] Finally, an important consideration when using Rozol is that mammals and birds directly consume the bait and succumb to chlorophacinone intoxication.[31,35] Placing the bait within the burrows of prairie dogs (as per the Rozol label) should reduce this hazard,[8] but it remains an important line of future research.

5 CONCLUSIONS

We conclude that the risk of secondary exposure to chlorophacinone residues by non-target animals consuming black-tailed prairie dogs exposed to the bait occurs within 27 days post-application of Rozol Prairie Dog Bait. Combined with the onset of intoxication from anticoagulant poisoning (i.e. lethargy), which increases susceptibility to predation, the highest period of risk is ≤14 days post-application and declines thereafter. These

results confirm that Rozol Prairie Dog Bait should not be used in areas where black-footed ferrets or other sensitive species occur.

ACKNOWLEDGEMENTS

We thank Buckley Air Force Base for providing us access to capture prairie dogs. We also thank the NWRC Analytical Chemistry Unit for analyzing the tissue samples. This project was funded by the United States Fish and Wildlife Service. The study was conducted under IACUC-approved Study Protocol QA-754. Mention of a commercial product or company does not represent an endorsement by the US government. We thank anonymous reviewers for their comments on this manuscript.

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