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RESEARCH ARTICLE

Toxicological responses to sublethal anticoagulant rodenticide exposure in free-flying hawks

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Abstract

An important component of assessing the hazards of anticoagulant rodenticides to non-target wildlife is observations in exposed free-ranging individuals. The objective of this study was to determine whether environmentally realistic, sublethal first-generation anticoagulant rodenticide (FGAR) exposures via prey can result in direct or indirect adverse effects to free-flying raptors. We offered black-tailed prairie dogs (*Cynomys ludovicianus*) that had fed on Rozol® Prairie Dog Bait (Rozol, 0.005% active ingredient chlorophacinone, CPN) to six wild-caught red-tailed hawks (RTHA, *Buteo jamaicensis*), and also offered black-tailed prairie dogs that were not exposed to Rozol to another two wild-caught RTHAs for 7 days. On day 6, blood was collected to determine CPN's effects on blood clotting time. On day 7, seven of the eight RTHAs were fitted with VHF radio telemetry transmitters and the RTHAs were released the following day and were monitored for 33 days. Prothrombin time (PT) and Russell's viper venom time confirmed that the CPN-exposed RTHAs were exposed to and were adversely affected by CPN. Four of the six CPN-exposed RTHAs exhibited ptiloerection, an indication of thermoregulatory dysfunction due to CPN toxicity, but no signs of intoxication were observed in the reference hawk or the remaining two CPN-exposed RTHAs. Of note is that PT values were associated with ptiloerection duration and frequency; therefore, sublethal CPN exposure can directly or indirectly evoke adverse effects in wild birds. Although our sample sizes were small, this study is a first to relate coagulation times to adverse clinical signs in free-ranging birds.

Keywords *Buteo jamaicensis* · Chlorophacinone · Coagulopathy · First-generation anticoagulant · Rodenticide · Prothrombin time · Ptiloerection · Rozol

Introduction

Black-tailed prairie dogs (BTPDs, *Cynomys ludovicianus*) are fossorial, communal rodents that range from south-central Canada to northeastern Mexico. Black-tailed prairie dogs in the USA and Mexico are an important prey for several species of raptors during fall migration and in winter,

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especially for the ferruginous hawk (*Buteo regalis*, Ng et al. 2020). However, BTPDs are considered a major pest species by many in the agricultural community and have been subjected to extensive eradication attempts from the early 1900s to the present day. These eradications are often promoted and conducted by county, state and federal agencies (Fox-Parrish 2012; Miller and Reading 2012; Vyas 2013; Vyas et al. 2012, 2017). Two first-generation anticoagulant rodenticides (FGARs), Rozol® Prairie Dog Bait (hereafter Rozol, winter wheat coated with 0.005% active ingredient chlorophacinone [CPN] and green dye) and Kaput®-D Prairie Dog Bait (0.005% active ingredient diphacinone), are registered for prairie dog control in the USA (https://liphatech.com/wp-content/uploads/2018/11/ENG_RZ_PrairieDogBait_Label-1.pdf; https://kaputproducts.com/wp-content/uploads/2013/07/72500-22-50lbKaput-D-Prairie-Dog-Label.pdf). First-generation anticoagulant rodenticides inhibit vitamin K epoxide reductase, disrupting blood clotting activity that can result in hemorrhage leading to death (Rattner et al. 2014b).

Raptor exposure to FGARs occurs after consumption of contaminated prey because 1) Rozol and Kaput®-D Prairie Dog Bait are registered for use during fall and winter when BTPDs can be a primary concentrated food source for diurnal raptors (Vyas et al. 2017); 2) mortality from FGARs may occur up to 3 weeks after exposure (Witmer et al. 2016), during which time, FGAR-contaminated BTPDs are available as prey; 3) depending on the size of a BTPD colony and the weather, FGAR-contaminated BTPDs may be available as prey for at least 4 weeks after FGAR application (Vyas et al. 2012); and 4) raptors forage preferentially on moribund BTPDs in FGAR-treated colonies instead of on active BTPDs in colonies not treated by FGARs (Vyas et al. 2017). These factors elevate potential secondary exposure and poisoning of raptors, but documenting adverse effects to free-ranging raptors is exceptionally problematic because: 1) exposure to and adverse effects of FGARs often occur on private land (Vyas et al. 2012) where public monitoring and detection are typically limited; 2) there are no systematic monitoring requirements to search for FGAR-affected non-target wildlife at BTPD colonies or in surrounding areas; 3) given the protracted time-course between FGAR exposure and mortality, raptors may die at locations distant from FGAR-treated BTPD colonies, thus reducing their association with the rodenticide application; 4) because of the relatively short FGAR half-lives in birds, the remnant residue concentrations in affected birds may not be detectable or may be too small to attribute the adverse effect to FGAR exposure (Rattner et al. 2015); and 5) raptors may succumb to FGARs indirect adverse effects (e.g., hypothermia, starvation, disease) that can confound identifying the underlying FGAR exposure. Because of these hurdles, only a few raptor mortalities have been attributed to chlorophacinone (CPN) exposure at BTPD colonies, and these mortalities were opportunistically discovered (Ron Klatsake, Executive Director Audubon of Kansas, personal communications; US Fish and Wildlife Service, 2012). The objective of this study was to determine whether environmentally realistic, sublethal FGAR exposures via prey can result in direct or indirect adverse effects to free-flying raptors.

Materials and Methods

Methods overview

We offered Rozol ad libitum to captive BTPDs for 19 days. The contaminated BTPDs were fed to six wild-caught red-tailed hawks (RTHAs, *Buteo jamaicensis*, CPN-exposed RTHAs), whereas BTPDs that were not exposed to Rozol were presented to two wild-caught RTHAs (reference birds) for 7 days. On day 6 of the RTHA exposure period, blood was collected from all eight RTHAs to determine chlorophacinone's effects on blood clotting time. On day 7 of the RTHA exposure period, the RTHAs were fitted with tail-mounted VHF radio telemetry transmitters and were released the following day. One reference bird (RTHA 3) received a leg injury during handling on the day before release and was taken to a wildlife rehabilitator for treatment. We used telemetry and visual observations to monitor RTHA survival, activity, and overt physiological condition for 33 days. Data collected included BTPD survival and hepatic CPN residues, and RTHA blood clotting time, body weights, and their fates after release.

Black-tailed prairie dog Rozol exposure

Forty-four BTPDs were live-trapped (CountyLine Catch and Release Live Traps, 81.3 cm × 30.5 cm × 25.4 cm) from a colony at the U.S. Fish and Wildlife Service Rocky Mountain Arsenal National Wildlife Refuge (hereafter, Refuge), Commerce City, Colorado, in January 2012. The Refuge has a well-documented history of contamination (U.S. Army 1996; Edson et al. 2011; https://www.epa.gov/fedfac/rocky-mountain-arsenal-site-spotlight-text-only-version). Before being dedicated as a wildlife refuge in 1992, the area was named the Rocky Mountain Arsenal and it was used by the U.S. Army and the Shell Chemical Company (1942–1982) to manufacture, store, and dump chemical warfare agents, incendiary munitions, insecticides, and herbicides. Consequently, parts of the Refuge soil and groundwater exceeded acceptable risks for human health and biota (U.S. Army 1996). In 1987, the U.S. Environmental Protection Agency listed the Rocky Mountain Arsenal on the National Priorities List under the Comprehensive Environmental Response, Compensation, and Liability Act (i.e., Superfund) that provides "for liability, compensation, cleanup, and emergency

response for hazardous substances released into the environment and the cleanup of inactive hazardous waste disposal sites" (42 U.S.C. § 9601 et seq.; https://www.law.cornell.edu/uscode/text/42/chapter-103/subchapter-I). In 2010, the remediation was completed, and the Refuge was removed from the National Priorities List (U.S. Army 1996; Edson et al. 2011; https://www.epa.gov/fedfac/rocky-mountain-arsenal-site-spotlight-text-only-version). The Refuge's background is of relevance because although our BTPDs were trapped on the Refuge, the BTPD colony was not located in its contaminated or remediated parts (U.S. Army 1996).

Traps were baited with Stockyards Ranch Supply Sweet Mix (Commerce City, Colorado), because of its palatability to BTPDs and because this feed was not supplemented with menadione that could possibly interfere with anticoagulant rodenticide (AR) toxicity (Rattner et al. 2014a). Trapped BTPDs were transported to a commercial warehouse (approximately 17 km from the Refuge) for the BTPD exposure period. Timer lights in the unheated warehouse approximated outdoor photoperiod and ambient weather conditions. Black-tailed prairie dogs were individually sprayed with a commercially available flea control product (active ingredient deltamethrin) while in traps. Two of the 44 BTPDs were euthanized and frozen to determine background hepatic CPN residue levels. The remaining 42 traps with BTPDs were placed next to each other on a wooden lattice structure that was raised 40 cm above the floor using cinderblocks. To minimize aggression between BTPDs, those that appeared amicable towards each other, indicating that they were likely from the same coterie (family group, Hoogland 1995), were later placed in the same test cage or in adjacent test cages (Little Giant Pet Lodge™ Rabbit Hutch, 61 cm × 61 cm × 41 cm). This was done to prevent aggression between BTPDs from different coteries. Black-tailed prairie dogs were accustomed to the cages and the warehouse for 2 weeks. During this time, BTPDs were fed Oxbow® Western Timothy Hay (*Phleum pratense*) ad libitum to mimic their natural diet and apple slices daily for moisture. The hay and apples did not contain menadione. Black-tailed prairie dogs were observed for injury and illness at least twice daily.

On day 1 of Rozol exposure, hay was removed from the cages and BTPDs were provided ad libitum Rozol in cup feeders for 19 days. However, beginning day 2, a small quantity of hay was provided daily to prevent diarrhea. Our Rozol exposure design was based on field observations at a Rozol-treated BTPD colony where bait could be seen from burrow entrances for up to 27 days after application and moribund BTPDs were observed for at least 29 days following the application (Vyas et al. 2012). We do not believe that hay provisioning during the Rozol exposure test created an environmentally unrealistic scenario. The Rozol label brackets the application timing from October to the following year's green-up in order to minimize competition for Rozol from natural foods (https://liphatech.com/wp-content/uploads/2018/11/ENG_RZ_PrairieDogBait_Label-1.pdf). Because of the protracted time-course between Rozol exposure and mortality, even Rozol-exposed BTPDs must consume natural foods. Additionally, BTPDs in untreated colonies often survive the winter by foraging on sparse vegetation (Nimish B. Vyas, personal observation). Although we did not measure Rozol consumption by BTPDs, their behavior and CPN hepatic residues confirmed the Rozol exposure.

Black-tailed prairie dogs were observed for signs of CPN toxicity at least two times a day. Those exhibiting severe toxicosis similar to the signs observed in the field (Vyas et al. 2012) were euthanized with CO_2. However, many of the surviving BTPDs were inactive (eyes closed for extended periods of time), and it was difficult to determine whether they were sleeping or moribund. To distinguish between these two conditions, inactive BTPDs were gently prodded with a wooden dowel. If they did not respond, they were picked up by hand with nitrile gloves. If the BTPD vigorously attempted to escape and did not exhibit overt signs of toxicosis, it was returned to its cage with the presumption that it was resting as it would in its burrow and thus would not be available as prey for raptors. If the BTPD responded only weakly to the disturbance or did not respond but was alive, it was considered to likely be vulnerable raptor prey and euthanized (Vyas et al. 2017). Black-tailed prairie dogs found dead or those euthanized at daily checks were immediately double-bagged and frozen. These BTPD carcasses were later thawed and subsequently offered to captive RTHAs (Materials and methods, section Red-tailed hawk chlorophacinone exposure).

Red-tailed hawk capture, housing, and care

Eight RTHAs were trapped in and around the Refuge in February 2012, using bal chatri traps with live wild-caught mice (Berger and Mueller 1959). Red-tailed hawks were immediately transported to the Refuge, weighed, tethered via jesses, double swivels and a leash (1.5 m), and held in temporary outdoor housing structures. The eight temporary outdoor housing structures, based on standard falconry techniques, were designed by J. Michael Lockhart to individually house the RTHAs and to afford them both mobility and protection (hereafter, Lockhart structures). Each Lockhart structure was comprised of two perches (V perch and semicircular shelf perch) mounted on back panels (plywood or particleboard, 1.2 m × 2.4 m, placed approximately 8 m across from each other) and a perlon rope (8 mm diameter) that ran between the back panels. Captured RTHAs were brought to the housing structures where each leash was tied, via a standard falconry knot, to the metal ring on the perlon rope (Fig. 1;

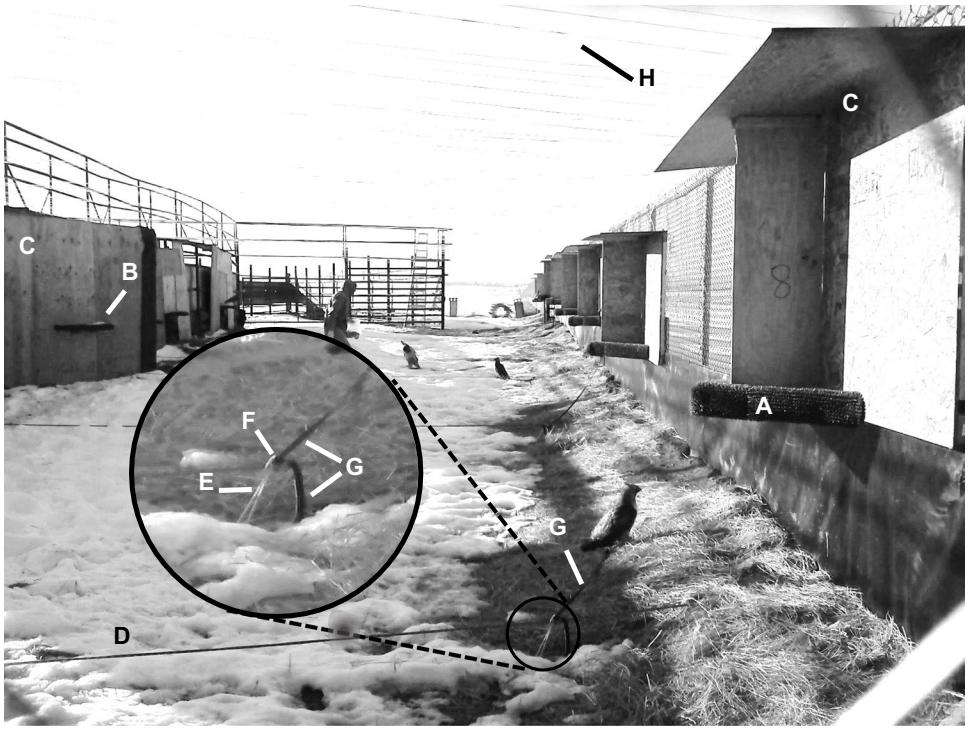

Fig. 1 Temporary Lockhart structures for housing red-tailed hawks (*Buteo jamaicensis*). A = V perch. B = Shelf perch. C = Back panel. D = Perlon rope. E = Bungie cord. F = Metal ring. G = Leash. H = Fishing line. Photo by Brian Fairchild, U.S. Fish and Wildlife Service

see detailed description of Lockhart structures in Online Resource 1).

Red-tailed hawks were accustomed to captivity for up to two weeks before beginning the RTHA exposure period. Each RTHA was inspected at least twice daily for overt signs of injury and or illness. While being accustomed to the pens, each RTHA was provided one-half of an uncontaminated BTPD daily. The unexposed wild-caught BTPDs were from the U.S. Fish and Wildlife Service's National Black-Footed Ferret Conservation Center, Carr, Colorado. Black-tailed prairie dogs were thawed overnight, and the next morning, carcasses were cut transversely below the rib cage such that the top half of the BTPD included the liver. The top half of one BTPD was presented to each RTHA by placing it on the V perch in the morning, and the uneaten portions were collected in the evening. The lower half of the BTPD was discarded.

Red-tailed hawk chlorophacinone exposure

Prior to starting the RTHA exposure period, two RTHAs were randomly assigned to a reference group (i.e., fed unexposed BTPDs) and six RTHAs were assigned to the CPN (active ingredient of Rozol) exposure group (i.e., fed BTPDs from the BTPD exposure period described in Materials and methods, section Black-tailed prairie dog Rozol exposure). While preparing BTPD carcasses for RTHA food, a small piece of liver (mean ± SD = 0.33 g ± 0.21 g) was collected and frozen from the unexposed BTPDs (n = 12) that were provided to the reference RTHAs and from the Rozol-exposed (n = 42) BTPDs for CPN residue analysis. Each BTPD carcass half was weighed before being presented to the RTHAs, and the uneaten carcass portion was weighed to estimate the amount consumed by each RTHA. By offering BTPD carcasses, we replicated the potential for secondary poisoning in RTHAs.

During the RTHA exposure period, all RTHAs were observed at least twice daily for signs of injury and toxicosis. Red-tailed hawks were weighed at the start and at the end of the exposure period (Table 1). Two-tailed t-tests for dependent means (https://www.socscistatistics.com/tests/ttestdependent/default.aspx) were conducted to compare the change in RTHA body weights during the exposure period. Because the reference group comprised of only two hawks, the body weights were analyzed for all eight birds as a group and also for the six birds that were provided Rozol-exposed BTPDs. Data were checked and met the assumptions of homogeneity (Levene's test, https://www.socscistatistics.com/tests/levene/default.aspx) and normality (Kolmogorov–Smirnov test of normality, https://www.socscistatistics.com/tests/kolmogorov/default.aspx) before conducting the paired t-tests.

Red-tailed hawk hematocrit and clotting time

On day 6 of the RTHA exposure period, a blood sample was collected from each RTHA (two reference RTHAs and six CPN-exposed RTHAs) to measure the effects of CPN on clotting time. First-generation anticoagulant rodenticides

Table 1 Red-tailed hawk (*Buteo jamaicensis*) information, coagulopathy and ptiloerection

Treatment	Red-tailed hawk	Origin	Sex	Mate	Initial Body weight (kg)	Body weight (kg) at release[d]	Hematocrit (%)	Fibrinogen (mg/dL)	Russell's viper venom time (seconds)	Prothrombin time (seconds)	Ptiloerection duration (days)[i]	Ptiloerection frequency (days)[j]	Number of high quality observation days[k]
Reference	SAVH[e]	R[a e]	F[b]	NA[c,l]	1.54	NA	40.0	229.9	20.4	20.4	NA	NA	NA
Reference	28	R[f]	U	NA	1.33	NA	22.2	164.1	11.4	13.8	NA	NA	NA
Reference	T	E[g]	F	NA	1.55	NA	43.3	157.2	28.6	14.1	NA	NA	NA
Reference	L	E[g]	F	NA	1.58	NA	45.5	160.2	21.8	15.1	NA	NA	NA
Reference	1	W,C[m]	M	F	1.03	1.24	44.4	187.6	22.4	12.35	0	0	15
Reference	3	W,C	F	None[h]	1.51	1.56	44.4	55.5	28.15	16.85	NA	NA	NA
Mean ± SD								159.1 ± 57.6	22.1 ± 6.38	15.4 ± 2.9			
CPN[n]	2	W,C	F	M	1.32	1.48	42.2	264.2	140.15	64.05	0	0	18
CPN	5	W,C	F	M	1.34	1.36	44.4	228.4	61.65	44.55	5	1	15
CPN	6	W,C	F	None	1.43	1.37	46.6	217.1	81.4	72.02	10	2	18
CPN	7	W,C	F	None	1.53	1.40	33.3	256.6	65.15	> 300	13	3	8
CPN	8	W,C	M	None	0.87	1.08	42.2	254.8	61.6	229.05	13	6	9
CPN	10	W,C	M	F	1.04	1.16	41.1	239.3	118.85	44.1	0	0	15

[a]Wild, bled under anesthesia. [b]Female based on weight. [c]RTHA origin not known and no initial weight. [d]The Rehabilitation and Education RTHAs were weighed once when collecting blood and the Captured (wild-caught for the study) RTHAs were weighed on the day of release. Additional reference blood samples were collected from RTHAs at: [e]South Arundel Veterinary Hospital, Edgewater, Maryland; [f]Birds of Prey Foundation, Broomfield, Colorado; and [g]Watkins Nature Center, Kettering, Maryland. [h]No mate observed during trapping and telemetry. [i]The time interval between when the RTHAs were released and the last day when ptiloerection was observed for each RTHA. [j]The number of days ptiloerection was observed during that time interval for each RTHA. [k]The number of days on which views allowed determination if RTHAs displayed ptiloerection. [l]not applicable. [m]captured from the wild for the study. [n]chlorophacinone

impair formation of fibrin (needed for creating blood clots), thereby prolonging the clotting time and increasing the chances of internal and external hemorrhaging (Rattner et al. 2014b). To increase the number of reference samples, blood was also collected from four other RTHAs that were either in rehabilitation or were in an education facility: RTHA 28 at the Birds of Prey Foundation, Broomfield, Colorado, RTHA SAVH at the South Arundel Veterinary Hospital, Edgewater, Maryland, and RTHAs T and L at the Watkins Nature Center, Kettering, Maryland (Table 1).

A 0.9 mL blood sample was drawn from the ulnar vein into a 1-mL syringe containing 0.1 mL of 3.2% sodium citrate. Red-tailed hawks were fully conscious, with the exception of one individual (RTHA SAVH) receiving rehabilitation treatment that was under isoflurane anesthesia. Citrated plasma samples were analyzed for fibrinogen (TCT) concentration, Russell's viper venom time (RVVT), and prothrombin time (PT) at U.S. Geological Survey Eastern Ecological Science Center in Beltsville, MD (Rattner et al. 2010, 2011; Hindmarch et al. 2019). Fibrinogen concentration was determined to confirm sample integrity, and then, the RVVT and PT assays were run to measure the effects of CPN on blood clotting times. Assay methods for TCT, RVVT and PT for use in several species of raptors have recently been described in detail (Rattner et al. 2011; Hindmarch et al. 2019; see Online Resource 2 for details). Using these methods, the 12 citrated RTHA plasma samples were assayed in duplicate with a mean coefficient of variation ± standard deviation of 2.61 ± 2.10% for TCT (n = 12), 6.16 ± 5.01% for RVVT (n = 12), and 4.40 ± 3.78% for PT (n = 11 excluding one sample that failed to clot in 300 s).

Red-tailed hawk telemetry, release and survival monitoring

On day 7 of the RTHA exposure period, RTHAs were fitted with tail-mounted VHF radio transmitters programed to double the signal rate to indicate mortality (Advanced Telemetry Systems). Thereafter, RTHAs were returned to their Lockhart structures. The next morning, RTHAs were weighed, and six CPN-exposed RTHAs and one reference RTHA were released at the Refuge. The second wild-caught reference RTHA (RTHA 3) received a leg injury during handling on the day before release and was taken to a wildlife rehabilitator for treatment. Her radio transmitter was removed, and she was taken for treatment at the Birds of Prey Foundation, Broomfield, Colorado. Reference RTHA 3 was excluded from field monitoring. The locations and survival of the seven released RTHAs were monitored over 33 days (February 19–March 22, 2012) using radio telemetry throughout the Denver, Colorado metropolitan area and the surrounding areas extending northward along the front range to Fort Lupton, eastward to Limon, and southward to Castle Rock.

Visual confirmations and the well-being of tagged RTHAs were made whenever possible. Some of our CPN-exposed birds exhibited ptiloerection (puffed-up feathers, a possible sign of CPNs direct or indirect adverse effects, Chaplin et al. 1984; Gobeli et al 2017; Hill et al. 1980; Kitulagodage 2011). We documented ptiloerection duration (time interval between when the RTHAs were released and the last day when ptiloerection was observed for each RTHA) and ptiloerection frequency (number of days ptiloerection was observed during that time interval for each RTHA). We used the Spearman's coefficient test (http://www.socscistatistics.com/tests/spearman/default2.aspx) to determine whether the observed frequency of ptiloerection was an artifact of observation bias. That is, assuming that all RTHAs exhibited the same frequency of ptiloerection, was the observed frequency of ptiloerection greater for RTHAs that provided us with a greater number of high quality views? We considered our observations to be of high quality if ptiloerection could be observed if it occurred, and low-quality observations were those where the RTHA was observed but it was too far away to determine whether it displayed ptiloerection. The high-quality observations occasionally afforded us views on the fullness of RTHAs' crops. We then tested for the association between RTHA body weight (change in body weight during the RTHA exposure period and body weight at release) and duration and frequency of ptiloerection. That is, were RTHAs with smaller body weights more likely to display ptiloerection than plumper hawks? Finally, we used Spearman's coefficient test to see whether there was an association between clotting time (PT and RVVT) and the duration and frequency of ptiloerection. That is, were the frequency and duration of ptiloerection associated with either the direct or indirect effects of CPN?

Black-tailed prairie dog chlorophacinone hepatic residue analysis

Liver samples from unexposed and Rozol-exposed BTPDs were analyzed for chlorophacinone concentrations at the U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, Maryland USA. Analysis methods have been previously described (Albert et al. 2010; Vyas et al. 2012) and provided in Online Resource 3. The lowest CPN standard (Sigma-Aldrich, St. Louis, MO, USA) used was 0.005 µg/ml. Two additional concentrations (0.001 and 0.05 µg/ml) were used to establish linearity and R^2 generally exceeded 0.990. The detection limit for the method was 0.01 µg/g. The average percent recovery for laboratory procedural spikes was 106%, while the average recovery for spiked tissue samples was 88%. The average relative percent

difference for duplicate sample analyses was 17.8%, and average total blank recoveries were 0.02 ug.

A two-tailed t-test for independent means (https://www.socscistatistics.com/tests/studentttest/default2.aspx) was conducted to compare hepatic CPN residue concentrations between CPN-exposed BTPDs that were euthanized and CPN-exposed BTPDs that were found dead during the BTPD exposure period. Data were checked and met the assumptions of homogeneity (Levene's test, https://www.socscistatistics.com/tests/levene/default.aspx) and normality (Kolmogorov–Smirnov test of normality, https://www.socscistatistics.com/tests/kolmogorov/default.aspx) before conducting the paired t-test.

Chlorophacinone residues were detected in livers of all unexposed BTPD (n = 13). Seven of these liver samples with the greatest CPN residue values were reanalyzed with three blank samples between each reference sample. The Grubb's Test for Outliers (GraphPad Prism version 6.04 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com) identified one sample (8.4 ug/g after first residue analysis and 5 ug/g after reanalysis) as an outlier, and it was excluded from further analyses. Descriptive statistics of CPN residues in livers from unexposed BTPDs (n = 12) were calculated (GraphPad Prism version 6.04 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). A paired t-test (https://www.socscistatistics.com/tests/ttestdependent/default.aspx) was used to compare CPN concentrations from the six control BTPD liver samples before reanalysis to the same six samples after reanalysis. A second paired t-test was conducted to compare CPN concentrations from all 12 control BTPD liver samples before six of the samples were reanalyzed to the 12 control samples that included six samples that were not reanalyzed and the six samples that were reanalyzed. Data were log-transformed to meet the assumptions of homogeneity (Levene's test and normality (Kolmogorov–Smirnov test), prior to the t-test.

Results

Black-tailed prairie dog Rozol exposure

Black-tailed prairie dogs offered Rozol exhibited overt signs of CPN exposure and intoxication throughout the 19-day exposure period. Signs of exposure and toxicosis included green coloration around the mouth and green droppings because of Rozol's dye, lethargy, weakness when walking, tremors while resting, lack of coordination, gurgling sound after alarm calling, reduction in alarm calling, moribundity, and mortality. Our anecdotal observations suggest that when some BTPDs exhibited adverse signs, they reduced or stopped feeding on Rozol until the severity of the overt signs was reduced and then resumed feeding on Rozol. The first BTPD to be euthanized during the BTPD exposure period was on day 5, and the first BTPD found dead was on day 8. Thirty-four BTPDs were euthanized because they were moribund and 8 BTPDs were found dead. No BTPDs were euthanized that were not moribund. The largest number of mortalities (euthanized and found dead, ~ 43% of the BTPDs) were recorded on days 12 and 13. As in the field (Vyas et al. 2012), the onset, intensity, and persistence of signs in the laboratory varied among individual BTPDs.

The physiological signs of Rozol exposure were incidentally observed in BTPD carcasses, while they were cut for presentation to RTHAs. These signs were consistent with the mode of action of ARs. Hemorrhage in the thoracic cavity was observed in at least 24 BTPDs, hemorrhaging in the abdominal cavity was noted in at least 24 BTPDs and rectal bleeding was apparent in at least five BTPDs. Furthermore, 11 BTPDs had green colored gastrointestinal tracts from Rozol's dye. Eighteen BTPDs showed one sign of exposure; 17 BTPDs two signs of exposure; four BTPDs three signs of exposure and two BTPDS did not show any of the above signs of exposure.

Black-tailed prairie dog chlorophacinone hepatic residues

Hepatic CPN residues in Rozol-exposed BTPDs (n = 42) ranged 0.09–10 ug/g, wet weight (w.w.). Hepatic residues were greater in BTPDs that were found dead (mean ± SD = 5.9 ug/g ± 3.6 ug/g, w.w., n = 8) than in those euthanized (mean ± SD = 3.7 ug/g ± 2.4 ug/g, w.w, n = 34; t(40) = -2.09, p = 0.04).

Chlorophacinone residues were detected in all liver samples from unexposed BTPDs that were presented to the reference RTHAs (mean ± SD = 1.62 ug/g ± 1.57 ug/g, w.w., range = 0.18 – 5.4 ug/g, w.w., n = 12). Six of these samples with the greatest CPN residues (mean ± SD = 2.70 ± 1.58 ug/g w.w., range = 1.0—5.4 ug/g, w.w., n = 6) that were reanalyzed with three blank samples between each sample, resulted in significantly smaller residue concentrations (mean ± SD = 0.80 ± 0.57 ug/g, w.w., range = 0.2 – 1.6 ug/g, w.w., n = 6; t(5) = -3.82, p = 0.01). Hence, CPN residues in the 12 reference livers from unexposed BTPD (six samples not reanalyzed + six samples reanalyzed) also significantly declined after reanalysis (mean ± SD = 0.67 ug/g ± 0.47 ug/g, w.w., range = 0.18 – 1.6 ug/g, w.w., n = 12, t(11) = -2.71, p = 0.02). Chlorophacinone residues in livers from the unexposed BTPDs (those provided by the National Black-Footed Ferret Conservation Center and the two BTPDs trapped at the Refuge as reference animals) are further addressed in Discussion section Red-tailed hawk chlorophacinone exposure and clotting time.

Red-tailed hawk chlorophacinone exposure

All eight (6 CPN-exposed and 2 reference) RTHAs appeared healthy and active during the RTHA exposure period and did not display overt signs of toxicosis. We do not have data for amount BTPD eaten on day 7 for reference RTHA 1 and reference RTHA 3 did not eat on the last day, likely because of her injury. Mean ± SD BTPD consumption/day for reference RTHAs was 103.76 g ± 45.76 g, n = 12 days (2 RTHAs × 6 days) and for CPN-exposed RTHAs was 120.07 g ± 36.82 g, n = 42 days (6 RTHAs × 7 days). Mean ± SD RTHA body weights before CPN exposure for all eight hawks and for the six CPN-exposed hawks were the same (1.26 kg ± 0.25 kg) and the mean ± SD RTHA body weights at release were 1.33 kg ± 0.16 kg for all eight hawks and 1.31 kg ± 0.15 kg for the six CPN-exposed hawks. Mean ± SD RTHA body weights at release were 1.16 kg ± 0.08 kg, n = 3, for males and 1.43 kg ± 0.08 kg, n = 5 for females. No significant differences were detected between body weights at the start of the CPN exposure period and at release for all eight hawks ($t[7] = -0.69$, $p = 0.49$) and for the six CPN-exposed hawks ($t[4] = 0.98$, $p = 0.37$).

Red-tailed hawk hematocrit and clotting time

Hematocrit of five of the six reference RTHAs ranged from 40.0 to 45.5%, with the remaining reference RTHA (RTHA 28) having a hematocrit of 22.2% (anemic) (Table 1). For five of the six RTHAs fed CPN-exposed BTPDs, hematocrit ranged from 41.1 to 46.6%, whereas a single RTHA exposed to CPN (RTHA 7) had a hematocrit of 33.3, borderline anemic (Table 1).

Fibrinogen concentrations for 11 of the 12 RTHAs ranged from 157.2 to 264.2 mg/dL, with the remaining RTHA (wild captured #3) having 55.5 mg/dL which nonetheless supported clotting in both the RVVT and PT assays (Table 1). Russell's viper venom time of reference RTHAs (mean ± SD) was 22.1 ± 6.28 s, but all CPN-exposed RTHAs exceeded this mean by more than 5 standard deviations (RVVT range 61.6 to 140.2 s). Likewise, PT of the six reference RTHAs was 15.4 ± 2.9 s, and values for all RTHAs fed CPN-exposed BTPDs also exceeded this mean by more than 5 standard deviations (PT range 44.1 to > 300 s). Lengthening of PT by more than two standard deviations above baseline values is suggestive of AR exposure (Rattner et al. 2014b).

Red-tailed hawk telemetry tracking and survival monitoring

Following the 7-day RTHA exposure period, seven RTHAs were released from captivity (one reference RTHA and six CPN-exposed RTHAs). Upon release, the RTHAs flew well and appeared not to be hindered by the attached transmitters. The Lockhart structures were within the territory of the RTHA 1 (reference group); his female mate visited him regularly during captivity. Upon his release, the pair flew together and landed on a nearby power pole (approximately 160 m away from release location) before flying away together.

During the 33-day RTHA survival monitoring period, RTHA 1 (reference group) and CPN-exposed RTHAs 2, 5, and 10 were observed perching, soaring, feeding, and displaying drop leg courtship with their mates, whereas no mates were observed for CPN-exposed RTHAs 6, 7 and 8 (Table 1). CPN-exposed RTHAs 5, 6, 7 and 8 intermittently displayed ptiloerection for 12 of the first 13 days after being released (Table 2); however, ptiloerection was not observed on those days for the single reference RTHA 1, CPN-exposed RTHAs 2 and 10 (Table 2) and other wild conspecifics in vicinity.

Table 2 Telemetry and visual detections of red-tailed hawks (*Buteo jamaicensis*) in Colorado during February and March 2012

Red-tailed hawk	Day after release																																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1	V	T	T	V	V	V	T	V	■	V	T	NS	NS	NS	NS	■	NS	T	V	■	■	V	V	V	V	V	V	■	■	V	V	T	T
2	T	V	V	V	V	V	T	V	■	V	V	T	NS	T	T	■	NS	V	V	■	■	V	V	V	V	T	V	■	■	V	V	T	V
5	V	V	T	V	P	V	T	V	■	V	T	T	NS	T	NS	■	T	V	V	■	■	V	V	V	T	V	V	■	■	V	T	V	V
6	V	V	T	V	V	P	T	V	■	P	V	V	V	V	NS	■	V	T	V	■	■	V	V	V	V	V	V	■	■	X	X	X	X
7	T	V	X	X	X	X	X	X	■	P	V	P	P	X	X	■	V	V	V	■	■	X	V	T	T	T	T	■	■	X	X	X	X
8	T	P	P	P	P	T	V	V	■	V	T	P	P	X	X	■	X	X	X	■	■	X	X	T	T	X	X	■	■	T	T	NS	T
10	V	V	V	T	V	T	T	V	■	V	T	V	V	T	NS	■	V	V	V	■	■	V	T	T	T	V	T	■	■	V	T	T	V

Hawks released on Day 0. V = Visual verification. T = Telemetry only verification. NS = Did not search for this hawk on this day because time was spent searching for hawks that had not been detected in recent searchers. P = Visual verification of ptiloerection. ■ = No monitoring conducted on this day. X = Hawks searched for, but no telemetry signal detected. Visual verification includes RTHA flying or perched far away such that ptiloerection could not be determined

Telemetry signals for every RTHA were not scanned on every day because effort often focused on RTHAs whose signals had not been detected during recent searches. Telemetry signals of reference RTHA 1 and CPN-exposed RTHAs, 2, 5 and 10 were detected on every day that we searched for them (Table 2). By contrast, telemetry signals were not detected for a total of 4, 13 and 9 days for CPN-exposed RTHAs 6, 7 and 8, respectively (Table 2). Despite considerable area and distant searching, no further contact was made with CPN-exposed RTHAs 6 and 7 after day 26 (Table 2). Telemetry signals from the reference RTHA 1 and CPN-exposed RTHAs 2, 5, 8 and 10 continued to be detected up to the last monitoring day (day 33) (Table 2).

CPN-exposed RTHA 5 was observed exhibiting ptiloerection on only 1 day, whereas CPN-exposed RTHAs 6, 7 and 8 were observed exhibiting ptiloerection for at least 2, 3 and 6 days, respectively. The reference RTHA 1 and CPN-exposed RTHAs 2 and 10 were not observed to exhibit ptiloerection during the RTHA survival monitoring period.

We found no association between the frequency of ptiloerection and the number of high-quality observations ($r = -0.56313$, $n = 7$, $p = 0.19$). No significant associations were found between the change in RTHA body weight while in captivity and the duration of ptiloerection ($r = -0.39$, $n = 7$, $p = 0.39$) or frequency of ptiloerection ($r = -0.28$, $n = 7$, $p = 0.54$). No significant associations were found between RTHA body weight on day of release and the duration of ptiloerection ($r = -0.05$, $n = 7$, $p = 0.90$) or frequency of ptiloerection ($r = -0.15$, $n = 7$, $p = 0.75$). However, there was a positive monotonic association between PT values and the duration of ptiloerection ($r = 0.88$, $n = 7$, $p = 0.01$; Fig. 2a) and between the PT values and the frequency of ptiloerection ($r = 0.85$, $n = 7$, $p = 0.01$; Fig. 2b). No significant associations between RVVT values and ptiloerection duration and frequency were detected ($r = -0.30$, $n = 7$, $p = 0.51$ and $r = -0.33$, $n = 7$, $p = 0.46$, respectively).

Discussion

General

This study resulted in two significant findings that are of relevance to understanding the risks of ARs to wild birds and are of importance to highlighting the utility of blood clotting time measurements in predicting adverse effects to birds. First, results showed that even sublethal chlorophacinone exposure can directly or indirectly evoke adverse effects in wild birds. Second, PT values, an indication of degree of coagulopathy, were positively associated with the duration and frequency of ptiloerection observed in free-ranging RTHAs.

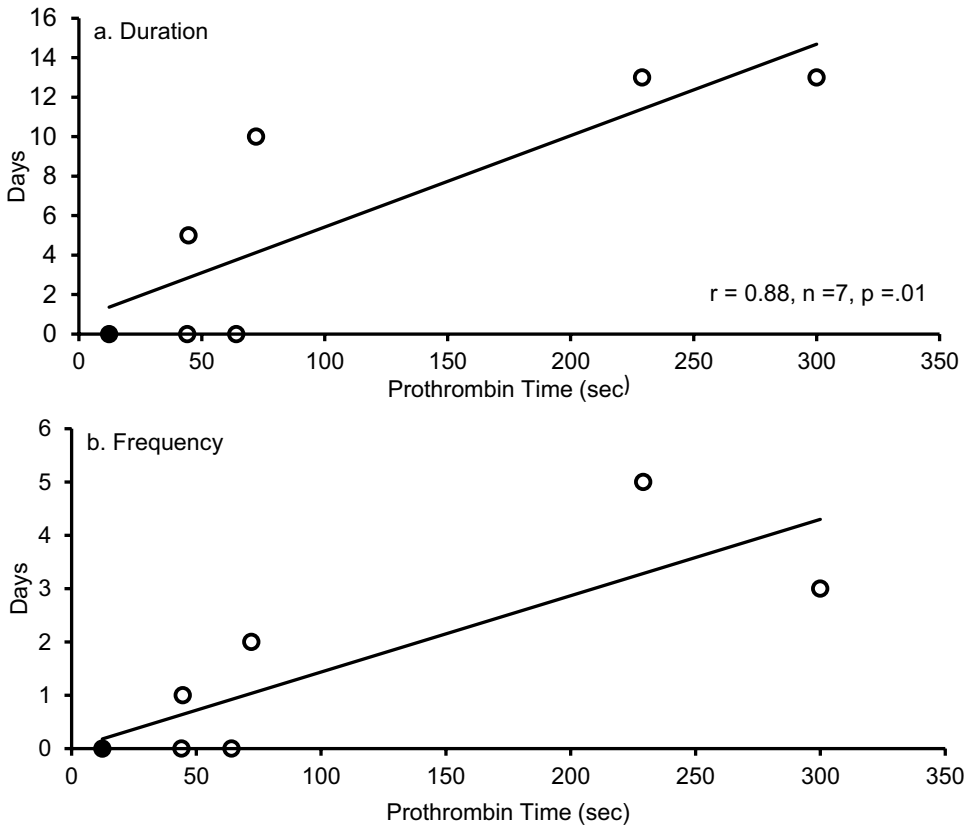

Fig. 2 Association between prothrombin time and ptiloerection duration (a last-day ptiloerection observed) and ptiloerection frequency (b number of days ptiloerection observed) for the reference ● and chlorophacinone-exposed ○ free-flying red-tailed hawks (*Buteo jamaicensis*)

Red-tailed hawk husbandry

Our CPN-exposed RTHAs showed no overt signs of adverse effects during the exposure period or on day of release. In general, several factors control the amount of food consumed per day and body weight in RTHAs, including energy expended, caloric content of prey, body condition and time of year (Preston and Beane 2020). Mean daily food consumption (reference RTHAs: 103.76 g ± 45.76 g and CPN-exposed RTHAs: 120.07 g ± 36.82 g) and mean body weights on day of release (females: 1.43 kg ± 0.08 kg and males: 1.16 kg ± 0.08 kg) were within reported range (Preston and Beane 2020; Tabaka et al. 1996). This is suggestive that RTHAs were healthy and consumed sufficient prey during captivity.

Red-tailed hawk chlorophacinone exposure and clotting time

We measured CPN residues in the BTPD liver subsamples that were collected before the BTPDs were presented to the RTHAs. We examined the relationship between hepatic residues and the occurrence of coagulopathy in our CPN-exposed RTHAs using the dietary-based toxicity reference values (dose–response curve) generated by Rattner et al. (2015) for American kestrels (*Falco sparverious*). The mean liver CPN concentrations of BTPDs that were found dead (5.9 ug/g, w.w.) or were euthanized (3.7 ug/g, w.w.) and the liver:total body concentration ratio of 5.2 (Shore and Coeurdassier 2018) were used to estimate the total body CPN concentrations of dead (1.13 ug/g, w.w.) and euthanized (0.71 ug/g, w.w.) BTPDs. Because RTHAs can encounter dead and live Rozol-exposed prey, the mean total body CPN concentration in BTPDs was estimated as 0.92 ug/g, w.w. From the mean body weight of CPN-exposed RTHAs on the day of release (1.3 kg) and the mean daily consumption by CPN-exposed hawks over 7 days (120 g), we estimate that the CPN-exposed RTHAs consumed 92.3 g/kg body wt.-day w.w. and that they were exposed to approximately 85 ug CPN/kg body wt.-day w.w. (92.3 g/kg body wt.-day w.w. × 0.92 ug/g, w.w.). The dietary-based toxicity dose–response curve predicted that if a population of RTHAs consumed approximately 85 ug CPN/kg body wt.-day w.w., approximately 80% of the RTHAs would experience prolonged PT and that 100% of the RTHAs would show extended RVVT. However, in our study, 100% of the RTHAs displayed prolonged PT. The greater than estimated RTHA response may simply be due to our small sample size or differences in sensitivities of the two species, but it may also reflect the differences in experimental methods. American kestrels were bred and experimented on in captivity, whereas the RTHAs were wild caught. The stressors of surviving in the wild (energetics of hunting, availability of food, nutritional quality of diet, weather, and general health) and the stress of being trapped and held in captivity may have increased our RTHAs' sensitivity to CPN. Therefore, the actual hazards of sublethal CPN exposure to wild birds may be greater than predicted by birds that are bred in captivity.

Clotting time measurements on blood collected on day 6 of the 7-day RTHA exposure period revealed coagulopathy in all CPN-exposed RTHAs when compared to the reference RTHAs. Based on the PT and RVVT values (Table 1), all CPN-exposed RTHAs lacked activated clotting factors to sustain coagulation at rates observed in the reference RTHAs. The prolonged PT and RVVT values of CPN-exposed RTHAs provided confirmation that these birds were exposed to CPN. Similarly, although CPN residues were detected in our reference BTPD livers, the significantly smaller PT and RVVT values of the reference RTHAs, within range of reference RTHAs in rehabilitation and educational facilities, provide evidence that the reference BTPDs presented to the reference RTHAs were likely not contaminated. We suspect the CPN residues in the reference samples were from carryover in the column during residue analysis. We base this on the fact that the second residue analysis of the six hepatic samples from unexposed BTPDs showed significantly smaller CPN residues than when analyzed without the three blank samples between each sample. Thus, the CPN concentrations in the reference samples could be attributed to residue carryover in the column during analysis.

Red-tailed hawk ptiloerection and survival

After the 7-day RTHA exposure period, seven RTHAs (one reference and six CPN-exposed) were released from their Lockhart structures and their fates were monitored for 33 days using radio telemetry and visual observations. Ptiloerection was the only observable variation in CPN-exposed RTHA status and behavior and was regarded as a possible indicator of general well-being of the hawks. Admittedly, this is nevertheless a subjective field assessment measure, as perched/roosting raptors can display the same behavior as well as raptors perched in very cold weather. However, we believe our observed ptiloerection events in CPN-exposed RTHAs were atypical behaviors and occurred at relatively disproportionate levels to normal, wild conspecifics. Four of the six CPN-exposed RTHAs were observed exhibiting ptiloerection for 1–6 days (total number of days on which ptiloerection was observed = 12 days) during the first 13 days of the RTHA survival monitoring period. It is important to note that the 13 days may represent the minimum number of days that these RTHAs exhibited ptiloerection because not all RTHAs could be directly observed daily. For example, visual contact was not made for RTHAs 7 and 8 (hawks with the most prolonged PT values; Table 1) on days 14–16 and on

days 14–23, respectively (Table 2). Furthermore, even when CPN-exposed RTHAs were observed, details such as ptiloerection could not always be discerned because the RTHAs were flying or were too far from the observer. Our free-flying CPN-exposed RTHAs may have also experienced additional adverse effects of CPN exposure such as those reported from toxicity studies with captive birds (e.g., bruising, change in body weight, prolonged clotting time, Radvanyi et al. 1988; Rattner et al. 2014a; Savarie et al. 1979). However, once our RTHAs were released from captivity, only striking overt signs of CPN exposure (e.g., ptiloerection) could be observed from a distance.

Possible cause of observed ptiloerection

Birds use considerable energy to preserve homeothermy in cold weather. One mechanism for preventing hypothermia is ptiloerection (Veghte 1964; Schwab and Schafer 1972; Hill et al. 1980). Ptiloerection is a normal process where birds puff their feathers to trap air to increase insulation and decrease body temperature heat loss. Although RTHAs are well adapted to maintain homeothermy despite short-term prey fluctuations and cold temperatures in winter (Carr and Lima 2012; Chaplin et al. 1984), ptiloerection can signify an underlying thermoregulation dysfunction caused by disease (Cleton et al. 2014; Amer and Mekky 2019), starvation (Chaplin et al. 1984), hypothermia (Carr and Lima 2012) and contaminant exposure (Decino et al. 1966; Zinkl et al. 1981; Gobeli et al 2017; Mineau and Tucker 2002; Kitulagodage 2011). During the RTHA survival monitoring period, the mean snow depth was 0.25 cm (an amount not expected to conceal or suppress prey activity) and the mean temperatures at Denver International Airport (16 km from the hawk holding pens) (maximum = 13.3 °C, minimum = -1.1 °C; https://www.wunderground.com/history/monthly/us/co/denver/KDEN/date/2012-2; https://www.wunderground.com/history/monthly/us/co/denver/KDEN/date/2012-3) were within the 39-year range of temperatures for February and March (maximum = 7.2 °C, minimum = -7.2 °C; maximum = 12.8 °C, minimum = -2.8 °C, respectively; https://www.currentresults.com/Weather/Colorado/Places/denver-temperatures-by-month-average.php). Thus, the observed frequency and duration of ptiloerection in the CPN-exposed RTHAs are not expected to have been evoked by weather conditions.

The frequency of ptiloerection observations was also not associated with observation quality. That is, the number of high-quality views per RTHAs did not influence the number of days when we observed ptiloerection. Additionally, no significant associations were found between RTHA body weight and the duration or frequency of ptiloerection. This suggests that the RTHA body weights during captivity did not affect their likelihood for exhibiting ptiloerection. However, the significant association between PT and the frequency and duration of ptiloerection leads us to suggest that the incidents of ptiloerection resulted from CPN's direct or indirect effects (e.g., reduced resilience to environmental stressors, possibly due to hemorrhaging and anemia).

Possible influence of individual red-tailed hawk behavior

While in captivity, RTHA body weights were within range of RTHAs reported by Tabaka et al. (1996) and Preston and Beane (2020), and none of our RTHAs exhibited ptiloerection. We suggest that our RTHAs maintained homeothermy in captivity because of the regular availability of food, access to shelter (Lockhart structures), and low energy expenditures. After being released from captivity, the stress of survival in the wild (e.g., energetics of hunting, availability of food, nutritional quality of diet) could have triggered the adverse effects of CPN (e.g., ptiloerection). Vyas et al. (2006) reported that environmental stressors can increase sensitivity to a toxicant in free-ranging birds by more than an order of magnitude when compared to birds tested under laboratory conditions.

Telemetry signals from RTHAs 1, 2, 5 and 10 (RTHAs with ≤ 1 day of observed ptiloerection) were detected every day that we searched for them. Visual confirmations revealed that, after release, the reference RTHA 1 and the CPN-exposed RTHAs 2, 5 and 10 repatriated their territories and rejoined their mates. By contrast, CPN-exposed RTHAs 6, 7 and 8 were considered to be overwintering non-territorial floaters because no telemetry signals were detected from CPN-exposed RTHAs 6, 7 and 8 for 4, 13 and 9 days, respectively. The absence of telemetry detection despite wide-ranging searches for the signals, supports our presumption that these three CPN-exposed RTHAs wandered in and out of our search area and therefore did not hold territory. Visual observations additionally confirmed that these three RTHAs were solitary.

The benefits of a territory (e.g., familiarity with prey distribution and density, availability of perches, and reduced competition) and possible direct or indirect provisioning and protection by the mate may lessen some of RTHAs' stressors and render them less vulnerable to the effects of sublethal CPN exposure. That is, non-territorial and solitary RTHAs would be more susceptible to CPN's direct and indirect effects because they endured additional stressors (e.g., greater energetic demands when searching for unpredictable and unreliable prey, escaping aggression from territorial RTHAs, and searching for shelter and roosting site) than the RTHAs with territory and mate. Although PT values of CPN-exposed RTHAs appear to the driver for ptiloerection frequency and duration, it is possible that, because non-territorial RTHAs were moving about more widely,

they may have increased likelihood of encountering other ARs and other contaminants, and potentially experience greater physiological and clinical adverse effects (Rattner and Mastrota 2018; Rattner et al., 2020). Exposures to certain contaminants (e.g., lead, other ARs) before capture may have contributed to their prolonged PT values and exposure to the contaminants may have contributed to further disrupting homeothermy that resulted in increased duration and frequency of ptiloerection. Chlorophacinone-exposed RTHAs 6, 7 and 8 (non-territorial and solitary) exhibited larger PT values and greater duration and frequency of ptiloerection than CPN-exposed RTHAs 2, 5 and 10 (territorial and mated). Based on PT values and behavioral observations, it could be possible that that stressors encountered by the non-territorial solitary RTHAs before being captured for this study may have increased their sensitivity to CPN as revealed by their prolonged PT values and RTHAs that experienced greater PT values would be more vulnerable to stressors after CPN exposure as revealed by their greater incidents of ptiloerection.

Although CPN-exposed RTHA 5 held territory and was with a mate, she was observed exhibiting ptiloerection on one day. Her PT value (44.55 s) was similar to or lower than PT values of the two CPN-exposed RTHAs with territories and mates (RTHA 2, 64.05 s and RTHA 10, 44.1 s) that did not exhibit ptiloerection (Table 1). It is possible that RTHA 5 displayed ptiloerection because she had paired with a considerably smaller male. Based on CPN-exposed RTHA 5, it is an intriguing prospect that even relatively modest increases in PT (compared to reference RTHAs) may be reflected in overt adverse effects (Table 1) from the absence of an effective mate.

Based on our anecdotal observations, we hypothesize that the CPN-exposed RTHAs with territories and mates may be less likely to exhibit ptiloerection than the non-territorial, solitary CPN-exposed RTHAs. Thus, the interaction of stressors and the sublethal CPN exposure in our study may have been sufficient to disrupt homeothermy and increase the duration and frequency of ptiloerection in the CPN-exposed RTHAs without territory and mate. During this study, CPN-exposed RTHAs exhibited ptiloerection even during relatively mild environmental conditions but under harsher environmental conditions (e.g., longer periods of colder temperatures and wind, reduction in prey availability, etc.), their survival may be compromised. We therefore hypothesize that the individual bird's behavior (e.g., holding territory and having a mate) dictates the outcome of the interactions between stressors and contaminant exposure. It is of note that the variations in individual behavior that may make a free-flying bird more susceptible to contaminant exposure and toxicity or make the contaminant-exposed birds more vulnerable to stressors than its conspecifics cannot be gleaned from laboratory studies nor predicted by risk assessments. Additional field research is needed to address this uncertainty.

Conclusions

Our results are the first to demonstrate a relation between coagulopathy and clinical signs in free-flying raptors and to highlight the importance of following such measurement endpoints with detailed observations for subtle but potentially life-threatening adverse effects in the field. Therefore, the key to gathering evidence of the lethal and sublethal hazards of CPN lies in conducting thorough field monitoring to examine the full range of FGARs' adverse effects on non-target species over time that is consistent with the mode of action and time course of adverse effects, associated environmental and contaminant stressors, species biology and individual behavior. Given the relatively short half-lives of FGARs, if a raptor were to succumb from the indirect effects (e.g., hypothermia during colder weather than experienced during this study) of sublethal FGAR exposure, the underlying cause of death may be classified as undetermined. It has been suggested that sublethal AR exposure may compromise survival by affecting [the bird's] 'condition' (e.g., lethargy could impair hunting, loss of body mass could reduce energy stores during winter), susceptibility to disease, resilience (e.g., recovery from nonfatal collisions, accidents and trauma), tolerance to extreme weather, and even sensitivity to other toxicants (e.g., lead that can result in anemia), and could exacerbate blood loss during molt" (Rattner et al. 2014b). It was also noted that based on the research available at the time, the "impaired condition hypothesis remains challenging to test and resolve" (Rattner et al. 2014b). Despite our small sample size, the results of our current study begin to address this important knowledge gap that is critical for characterizing the hazards and risks of ARs.

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Authors' contributions Nimish B. Vyas did conceptualization, methodology, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, visualization, supervision, project administration, funding acquisition. Barnett A. Rattner was involved in methodology, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, visualization. J. Michael Lockhart contributed to methodology, formal analysis, investigation, resources, writing—review and editing, visualization. Craig S. Hulse and Clifford P. Rice did methodology, formal analysis, investigation, resources, writing—original draft, writing—review and editing. Frank Kuncir performed investigation and writing—review and editing. Kevin Kritz done investigation, writing—review and editing and funding acquisition. All authors read and approved the final manuscript.

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Data availability Citation for data release will be available after journal acceptance.

Declarations

Ethics approval and consent to participate All procedures involving animals were reviewed and approved by the U.S. Geological Survey, Eastern Ecological Science Center's Institutional Animal Care and Use Committee [Study plan title: Characterization of Avian Hazards Following Chlorophacinone (Rozol®) Use for Prairie Dog Control].

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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