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PRELIMINARY EVIDENCE OF ANTICOAGULANT RODENTICIDE EXPOSURE IN AMERICAN KESTRELS (*FALCO SPARVERIUS*) IN THE WESTERN UNITED STATES

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ABSTRACT.—Although there is extensive evidence of declines in the American Kestrel (*Falco sparverius*) population across North America, the cause of such declines remains a mystery. One hypothesized driver of decline is anticoagulant rodenticide (AR) exposure, which could potentially cause mortality or reduced fitness. We investigated AR exposure in wild American Kestrels in Utah, USA. We collected and tested for AR residues in liver samples ($n=8$) from kestrels opportunistically encountered dead and in blood samples ($n=71$) from live wild kestrels, both nestlings and adults. We found high detection rates in both tissues. Adult kestrels were more likely to exhibit exposure than juveniles sampled in nests. Three-quarters (six of eight) of tested liver samples from adult kestrels exhibited evidence of AR exposure. Additionally, liver samples ($n=19$) opportunistically collected from seven species of raptors within our study area had detectable levels of AR residues, with seven of eight raptor species evidencing exposure; across all raptors, five ARs were detected in liver samples, with brodifacoum the most prevalent, being found in over half (14 of 27) of samples. Over half (7 of 12) of the blood samples from adult kestrels had detectible levels of ARs, while only one of 59 juvenile nest samples tested positive. The difference in exposure rates between adults and juveniles could indicate differential exposure pathways by age class. Based on these findings, we recommend that ARs be further investigated as a potential cause of kestrel declines. Future research could focus on expanding sampling to provide sufficient sample sizes to test for potential nonlethal effects of AR exposure (e.g., fecundity, nesting success), identifying potential exposure pathways, and developing methods for passive sampling of ARs in excreta.

KEY WORDS: *American Kestrel*; Falco sparverius; *anticoagulant rodenticide*; *raptor conservation*; *toxicology*; *Utah*.

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EVIDENCIA PRELIMINAR DE EXPOSICIÓN A RODENTICIDAS ANTICOAGULANTES EN *FALCO SPARVERIUS* EN EL OESTE DE ESTADOS UNIDOS

RESUMEN.—Aunque existe una amplia evidencia de disminuciones en la población de *Falco sparverius* en América del Norte, la causa de tales disminuciones sigue siendo un misterio. Un responsable hipotético de la disminución es la exposición a los rodenticidas anticoagulantes (RA), que podrían causar mortalidad o reducir la condición física. Investigamos la exposición a los RA en individuos silvestres de *F. sparverius* en Utah, EEUU. Recolectamos y analizamos residuos de RA en muestras de hígado ($n = 8$) recolectadas de manera oportunista de individuos de *F. sparverius* encontrados muertos y en muestras de sangre ($n = 71$) de individuos silvestres vivos, tanto crías como adultos. Encontramos altas tasas de detección en ambos tejidos. Los individuos adultos fueron más propensos a exhibir exposición que los juveniles muestreados en los nidos. Tres cuartas partes (seis de ocho) de las muestras de hígado analizadas de individuos adultos exhibieron evidencia de exposición a RA. Además, las muestras de hígado ($n = 19$) recolectadas de manera oportunista de siete especies de aves rapaces dentro de nuestra área de estudio tuvieron niveles detectables de residuos de RA, con siete de ocho especies de aves rapaces evidenciando exposición; en todas las rapaces se detectaron cinco RA en las muestras de hígado, siendo el brodifacoum el más frecuente, encontrándose en más de la mitad (14 de 27) de las muestras. Más de la mitad (7 de 12) de las muestras de sangre de los individuos adultos tuvieron niveles detectables de RA, mientras que sólo una de las 59 muestras de juveniles en los nidos dio positivo. La diferencia en las tasas de exposición entre adultos y juveniles podría indicar vías de exposición diferenciales por clase de edad. Con base en estos hallazgos, recomendamos que los RA se investiguen más a fondo como una posible causa de la disminución de *F. sparverius*. Las futuras investigaciones podrían enfocarse en expandir el muestreo para proporcionar tamaños muestrales suficientes para evaluar los posibles efectos no letales de la exposición a los RA (por ejemplo, fecundidad, éxito de anidación), identificar posibles vías de exposición y desarrollar métodos para el muestreo pasivo de RA en los excrementos.

[Traducción del equipo editorial]

INTRODUCTION

The American Kestrel (*Falco sparverius*), North America's smallest falcon, has undergone long-term population declines across much of its northern range (Smallwood et al. 2009, McClure et al. 2017). Although the signals for decline are clear, the causes are uncertain. Hypothesized drivers include loss and alteration of habitat on breeding or wintering grounds, pathogens, loss of or competition for nesting cavities, increased predation by Cooper's Hawks (*Accipiter cooperii*), and lethal and sublethal adverse effects of chemicals and pesticides, especially insecticides and rodenticides (McClure et al. 2017).

Anticoagulant rodenticides (ARs) are widely used to control rodent pests. Widespread unintentional AR exposure of nontarget wildlife has been documented on a global scale (van den Brink et al. 2018). Raptors, including both diurnal and nocturnal species, are particularly vulnerable to AR exposure because they frequently consume rodents. For example, 11% of the Great Horned Owl (*Bubo virginianus*) population in Canada is estimated to be at risk of mortality from AR exposure (Thomas et al. 2011) and 100% of Red-tailed Hawks (*Buteo jamaicensis*) admitted to a wildlife clinic in Massachusetts had detectable levels of ARs in liver tissue (Murray 2020), along with other such studies (van den Brink et al. 2018). The Eurasian Kestrel (*Falco tinnunculus*), which is closely related to the American Kestrel and fills a similar ecological niche, had high rates of exposure in samples collected between 1997 and 2012 compared to other raptor species in the United Kingdom (Walker et al. 2011, Roos et al. 2021), indicating that kestrels may be at an elevated risk of exposure among raptors. Using these data, a recent analysis demonstrated a negative correlation between a kestrel population index and AR residue concentrations found in deceased kestrels in the same year, suggesting that ARs are potentially a population-limiting factor for the Eurasian Kestrel (Roos et al. 2021). Nonetheless, little is known about AR exposure in live, wild raptors, including American Kestrels, nor the population-level effects of AR exposure in raptors generally, including lethal and sublethal effects on demographics (Rattner et al. 2014).

Herein we report on initial steps to investigate AR exposure in American Kestrels (and other raptors) in the western United States to evaluate one of the primary hypothesized drivers of decline in this species. To do so, we sought to answer the following

questions: (1) Are raptors generally and kestrels specifically exposed to ARs within the study region? and (2) What are the best methods for evaluating kestrel exposure to ARs that will facilitate investigating lethal and sublethal responses? To address these questions, we collected liver and blood samples from kestrels over two breeding seasons (2019–2020). We also collected liver samples from other raptor carcasses that were opportunistically encountered within the study area. We then tested for associations between kestrel AR exposure and age, sample type, and habitat. Finally, we provide some suggestions for future research on this issue, with the aim of moving from documentation of exposure to quantifying potential population-level responses.

METHODS

Study Area and Sample Population. We sampled wild kestrels within the framework of HawkWatch International's (HWI) long-term study of American Kestrel demography in northern Utah (https://hawkwatch.org/our-work/kestrels). Professional biologists and community scientists (approximately 60 individuals/yr) monitor approximately 500 nest boxes along the Wasatch Front (i.e., the greater Salt Lake City metropolitan area) during the breeding season (March–July) annually. On average, 100 kestrel pairs nest in boxes each year, enabling the study of kestrel demography and factors influencing population stability. The study area, a 130-km-long north–south corridor between Ogden and Provo, Utah, USA (Fig. 1), encompasses a variety of landscapes. For this study, the habitat of each sampling location was classified as either urban, agricultural, or wildland, by an in-person evaluation augmented by a review of satellite imagery from Google Earth of the dominant characteristics in a 500-m radius around the sampling location. Urban areas had >50% coverage of buildings, roads, parking lots, and other urban infrastructure, but also some coverage of urban green spaces, such as parks, road margins, or lawns. Agricultural areas had >50% coverage of agricultural crops or intensively grazed livestock pastures. Wildlands had >50% coverage of undeveloped land or land managed for wildlife.

Sample Collection. We collected liver samples from dead kestrels and other raptors opportunistically found within the study area and blood samples from live adults and nestlings at nest boxes within monitored territories within the study area. We expected the two sampling techniques to have strengths and weaknesses and by collecting a variety of samples we sought to maximize the likelihood of AR detection. Because the half-life of some ARs in blood is relatively short (days to weeks; see Horak et al. 2018), this sampling methodology was chosen to inform about exposure at a specific geographic location (i.e., foraging territory around the nest) over a relatively short period of time. In contrast, liver sampling provides an assessment of exposure over a more protracted period (days to months), as ARs are retained in the liver longer than in blood. We collected liver samples opportunistically and thus we could not control the quantity of samples or their geographic distribution.

Opportunistically discovered dead kestrels within the study area were placed in a Ziploc bag, and the date, GPS location, and ID (if present, nest or band number) were noted. The bird was stored in a freezer (–20°C) as soon as possible on the same day as discovery. The Wildlife Rehabilitation Center of Northern Utah (WRCNU) collected additional carcasses, which originated in the general study area of northern Utah and were either brought to the facility dead or were from birds that died in captivity within one week of being submitted to the center. These samples were similarly labeled and immediately frozen (–20°C).

We collected blood samples during nestling banding visits, which took place within 1 wk of anticipated fledging (based on observations of egg laying and hatching dates). To do so, we plugged nest-box holes and then hand-removed nestlings from the box. Blood from either the basilic (wing) or medial metatarsal (leg) vein was drawn using 27-gauge untreated butterfly (winged) needles with attached catheter tubing to a syringe. We obtained up to 1 mL from each individual (<1% body mass), but because of the small size of the nestlings and resulting difficulty in venipuncture, often sample volume was smaller. For each nest, we continued to process 1–4 individuals until we collected 0.5–1 mL of blood total. Nestlings were returned to their nest box after hemostasis. For nestlings, blood from multiple individuals from the same nest was pooled in an untreated storage vial, such that each pooled sample was a sample of the nest, not the individual. We hereafter refer to these samples as "juvenile nest samples." For adults, we also opportunistically trapped adult kestrels in the vicinity of nest-boxes, including of adults attending nests where juvenile nest samples were collected. We followed the same methodology as for nestlings, except we did not pool

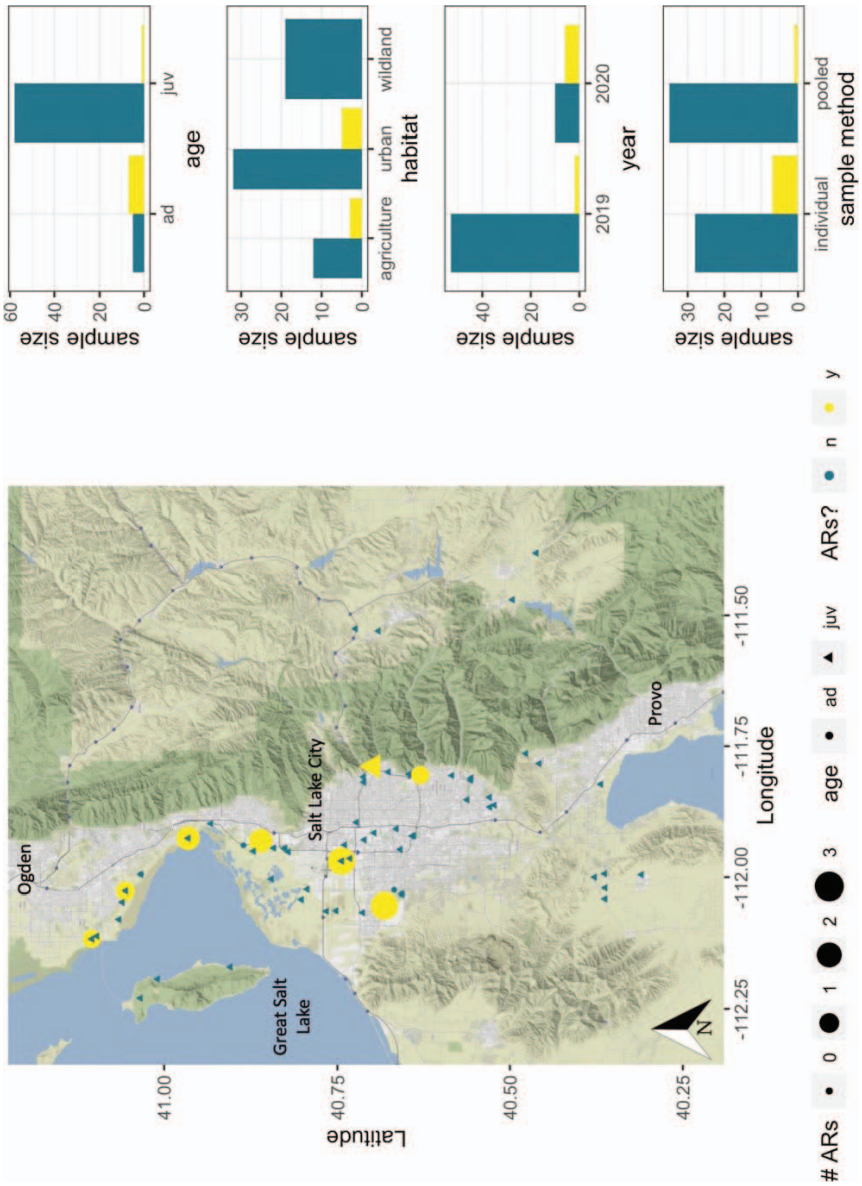

Figure 1. Overview of anticoagulant rodenticide (AR) sampling locations and patterns in exposure from American Kestrel blood sampling. The size of the sample point is indicative of the number of ARs found in the sample, the shape indicates the age, and the color indicates whether ARs were present. For age, ad = adult, juv = juvenile; for ARs, n = non-detect, y = detect.

samples. We labeled samples with date, nest ID, and band number and immediately placed them on ice while in the field and stored them in a freezer (–20°C) at the end of the day.

Reference blood samples from a captive colony of American Kestrels at the US Geological Survey (USGS) Eastern Ecological Science Center at the Patuxent Research Refuge (hereafter EESC) were collected to characterize and validate AR analytical methods. To do so, six adult American Kestrels (three males and three females) propagated from a captive colony at EESC were housed individually for 2 wk in small outdoor cages with a shade roof, perches, food tray, and water bowl, and fed dead laboratory mice (*Mus musculus*; Rattner et al. 2020). At the end of the 14-d period, each bird was weighed, a jugular venipuncture sample was collected in a heparinized syringe (approximately 1 mL or a volume equivalent to 1% of the bird's body weight), and the whole blood was transferred to a cryovial and frozen at –80°C. Additionally, liver tissue from three kestrels that were found dead at the breeding colony were collected and frozen in cryovials.

Our overall project design and field sampling methodology were reviewed and approved by the National Zoological Park Institutional Animal Care and Use Committee (NZP-IACUC, Proposal #19-29). Further, methods for control sample collection from the captive population of kestrels at EESC was reviewed and approved by the EESC-IACUC (Project 2020-01). HWI staff trapped, banded, and collected all kestrel blood and liver samples in the field, with authorization from the USGS Bird Banding Lab (Permit #21384).

Laboratory Analyses. All samples were shipped overnight on dry ice to the Analytical Chemistry Laboratory of the US Department of Agriculture Animal and Plant Health Inspection Service's (USDA-APHIS) National Wildlife Research Center (hereafter NWRC) for analysis. NWRC tested liver and blood samples for a suite of ARs (pindone, coumatetralyl, coumafuryl, warfarin, diphacinone, coumachlor, chlorophacinone, difenacoum, brodifacoum, bromadiolone, difethialone, flocoumafen) and desmethyl bromethalin (the primary metabolite of the neurotoxin bromethalin). Methods were developed for and validated using control American Kestrel liver and whole blood samples provided by EESC (Supplemental Material Table S1, S2).

Liver samples were analyzed using a version of a previously described method (Franklin et al. 2018), described in brief here. Homogenized liver (100 mg) was weighed into microcentrifuge tubes, fortified with deuterium-labeled internal standards of eight rodenticides to serve as surrogate analytes throughout the extraction, and extracted with acetonitrile (ACN). A dispersive solid-phase extraction (dSPE) clean-up procedure was used to prepare the sample for analysis via ultra-performance liquid chromatography (UPLC) on a C_{18} column coupled to a tandem mass spectrometer (MS/MS) with electrospray ionization (ESI) source. The peak area response ratio of each analyte relative to their respective internal standard was used to quantify each rodenticide. The detection limit (DL) is the lowest concentration of rodenticide in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit (QL) is the lowest concentration of rodenticide that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL for each rodenticide. This was performed by comparing the rodenticide response observed in control matrix fortified at the lowest quality control fortification level with the baseline noise observed at the retention time of each rodenticide in control matrix. The DL and QL are defined as the rodenticide concentration corresponding to S/N ratios of 3 and 10, respectively (Table S1).

Whole blood was analyzed using the same conditions described above for liver, but with the following modifications: whole blood was weighed into extraction vials to accommodate the varying consistency of the samples. It was then transferred to a microcentrifuge tube, surrogate analytes were added, then water, ACN, and NaCl were added, and then a portion of supernatant (CAN) reduced to dryness. No dSPE procedure was necessary. Samples were analyzed as described above (Table S2).

We report the detection, concentration, DL, and QL for each of thirteen rodenticide compounds from each of the samples. If no rodenticide was detected by the data acquisition software or if the observed concentration was less than the method DL, the rodenticide was reported as not detected (ND). We confirmed the identity of each rodenticide by the method acceptance criteria for retention time (±2%), qualifier-to-quantifier response ratio percent match (±30% for bromadiolone and difethialone; ±20% for all others), and surrogate analyte percent recovery (d_4-diphacinone $\geq$ 20%; d_4-coumatetralyl $\geq$ 30%; d_5-warfarin, d_4-difenacoum, d_4-brodifacoum, d_4-difethialone, and d_5-bromadio-

lone A ≥ 50%; d_5-bromadiolone B ≥ 60%). Results that failed any of these acceptance criteria were reported as ND. Concentration values >DL but <QL are indicative of an AR detection, but should be interpreted with caution, as the variability will be greater than the acceptable method performance.

NWRC analyzed three replicates of each sample when sample volume was adequate. Average concentrations determined from a combination of observed values and non-detects is calculated as follows: if all replicates were ND, then the average is reported as ND. If at least one replicate had a concentration >DL then an average residue was determined using the convention of substituting ½ DL for ND. We report the average concentration values.

Statistical Methods. Detection frequency, concentration range and central tendency were generated using descriptive methods for each AR and the summed concentration of second generation ARs. For AR residues >DL in some (>50%) but not all samples, central tendency was estimated using the Kaplan-Meier method and also by estimating the potential range of the mean (i.e., <DL values first assigned 0.00001 μg/g and lower limit mean calculated for group, followed by <DL values assigned the DL and an upper limit mean calculated for group; Helsel 2005). To examine patterns of AR exposure, we tested if there was an association between a binary positive detection (yes = 1 if >DL; no = 0 if <DL) of any AR in kestrel blood with age (adult or juvenile), habitat (urban, agricultural, wildland), year (2019, 2020), and sampling methodology (individual, pooled). To do so, we used generalized linear regression, specifying a binomial distribution. All statistical analyses were conducted using R Version 3.6.1 (R Core Team 2016) including the *NADA* package (Lee 2020).

RESULTS

We obtained 34 raptor liver samples in 2019, including samples from 15 American Kestrels and 19 individual raptors of other species. Of these, twelve kestrels and one Cooper's Hawk were opportunistically collected when encountered dead in the field by HWI staff and the remaining birds were collected by WRCNU. Seven kestrel carcasses had no identifiable liver due to their advanced state of decomposition, resulting in eight kestrel livers and 19 livers from other raptors (27 total) available for analysis (Table 1). Diphacinone, the only first generation AR detected, was present in one of eight kestrel liver samples and one of two Western Screech-Owl (*Megascops kennicottii*) samples (Table 1). Second generation ARs (brodifacoum, bromadiolone, difenacoum, and difethialone) were detected in the liver samples in five of eight (62%) American Kestrels, six of seven (86%) Great Horned Owl samples, two of three (67%) Red-tailed Hawks, two of three (67%) Cooper's Hawks, both (100%) Western Screech-Owl samples, one of two (50%) Merlin (*Falco columbarius*) samples, and the single Swainson's Hawk (*Buteo swainsoni*; 100%), but not in the single Sharp-shinned Hawk (*Accipiter striatus*; 0%) sample. Often several compounds were detected in a liver sample (Table 1).

We collected 73 whole-blood samples (57 in 2019 and 16 in 2020 with no repeated sampling of the same individual) from live, wild kestrels, including both adult and juvenile nest samples (which included blood from one to four juveniles within a nest). Two samples had insufficient volume, resulting in 71 kestrel samples for analysis. Eight of 71 (11%) kestrel blood samples had detectable levels of ARs with a range of one to three compounds (bromadiolone, brodifacoum, and difethialone) found in the positive samples (Table 2). Quantifiable levels of bromadiolone (>QL) were observed in five samples, brodifacoum in four samples, and difethialone in one sample. Blood concentration for positive detections ranged from 0.57 ng/mL to 18.8 ng/mL.

Adult kestrels ($n = 12$) were more likely to have a detectable AR exposure in blood than juvenile nest samples ($n = 59$; $t = -5.543$, df = 66, $P < 0.001$; Table 3), when controlling for habitat, year sampled, and sample methodology (i.e., individual or pooled sample). We found no differences among other variables (Table 3). Only one of 59 (1.7%) juvenile nest samples had detectable ARs in blood, whereas seven of 12 (58.3%) adults had detectable ARs in blood. Blood AR detection occurred principally along the periphery of the urban areas, in both urban and agricultural settings, but not in wildlands (Fig. 1).

DISCUSSION

We found evidence to support that raptors generally and kestrels specifically are exposed to ARs within our study region. There were positive detections in three-quarters of liver samples (six of eight) from opportunistically collected dead kestrels and over half of blood samples (seven of 12) collected from adult kestrels. In addition, liver

Table 1. Anticoagulant rodenticide (AR) detections in raptor liver samples (μg/g wet weight) from raptors opportunistically encountered dead within the study region of northern Utah in 2019. Values presented for each anticoagulant rodenticide are number of detections/sample size, arithmetic mean concentrations of detects, and Kaplan-Meier (KM) mean and estimated extremes of the mean if more than half of a species' samples had values >DL and more than one sample had a value >DL. ∑SGAR mean is the arithmetic mean concentration of all second-generation ARs that were >DL of each species. ∑SGAR range is the range of values of all second-generation ARs by species. ND indicates a "non-detection" of a particular compound. "-" indicates that there was no information for that value.

		First Generation AR	Second Generation ARs				
		Diphacinone	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	∑SGAR
Common Name (Scientific name)	n	Detects/N Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 μg/g	Detects/N Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 μg/g	Detects/N Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 μg/g	Detects/N Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 μg/g	Detects/N Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 μg/g	Detects/N Mean Conc. of Detects Conc. Range Detects >0.1 μg/g
American Kestrel	8	1/8	5/8	1/8	1/8	2/8	5/8
(*Falco sparverius*)		-	0.1094	-	-	0.2356	0.2224
		-	(0.0691; 0.0684–0.0691)	-	-	-	
		<DL–0.276	<DL–0.194	<DL–0.0925	<DL–0.0010	<DL–0.470	<DL–0.664
		1/8	3/8	0/8	0/8	1/8	3/8
Red-tailed Hawk	3	0/3	1/3	1/3	0/3	1/3	2/3
(*Buteo jamaicensis*)		-	-	-	-	-	0.0220
		-	-	-	-	-	
		ND	<DL–0.0108	<DL–0.0045	ND	<DL–0.0286	<DL–0.0286
		0/3	0/3	0/3	0/3	0/3	0/3
Swainson's Hawk	1	1/1	1/1	1/1	0/1	0/1	1/1
(*Buteo swainsoni*)		-	-	-	-	-	-
		-	-	-	-	-	
		0.0185	0.0067	0.0074	ND	ND	0.0141
		0/1	0/1	0/1	0/1	0/1	0/1
Sharp-shinned Hawk	1	0/1	0/1	0/1	0/1	0/1	0/1
(*Accipiter striatus*)		-	-	-	-	-	-
		-	-	-	-	-	
		ND	ND	ND	ND	ND	ND
		0/1	0/1	0/1	0/1	0/1	0/1
Cooper's Hawk	3	0/3	1/3	2/3	0/3	1/3	2/3
(*Accipiter cooperii*)		-	-	0.0088	-	-	0.0333
		-	-	(0.0065; 0.0059–0.0060)	-	-	
		ND	<DL–0.0456	<DL–0.0159	ND	<DL–0.0034	<DL–0.0649
		0/3	0/3	0/3	0/3	0/3	0/3

Table 1. Continued.

		First Generation AR	Second Generation ARs				
		Diphacinone	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	∑SGAR
Common Name (Scientific name)	*n*	Detects/*N* Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 µg/g	Detects/*N* Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 µg/g	Detects/*N* Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 µg/g	Detects/*N* Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 µg/g	Detects/*N* Mean Conc. of Detects (KM Mean; Extremes) Conc. Range Detects >0.1 µg/g	Detects/*N* Mean Conc. of Detects Conc. Range Detects >0.1 µg/g
Merlin	2	0/2	0/2	0/2	0/2	1/2	1/2
(*Falco columbarius*)		-	-	-	-	-	-
		-	-	-	-	-	
		ND	ND	ND	ND	<DL–0.0011	<DL–0.0011
		0/2	0/2	0/2	0/2	0/2	0/2
Western Screech-Owl	2	1/2	2/2	1/2	0/2	1/2	2/2
(*Megascops kennicottii*)		-	0.1705	-	-	-	0.1848
		-	-	-	-	-	
		<DL–0.0029	0.110–0.231	<DL–0.0052	ND	<DL–0.0234	0.1386–0.2310
		0/2	2/2	0/2	0/2	0/2	2/2
Great Horned Owl	7	0/7	4/7	5/7	0/7	3/7	6/7
(*Bubo virginianus*)			0.0293	0.0274	-	0.0286	0.0566
			(0.0196; 0.0167–0.0176)	(0.0203; 0.0195–0.0196)	-	-	
		ND	<DL–0.0653	<DL–0.0656	ND	<DL–0.0755	<DL–0.1535
		0/7	0/7	0/7	0/7	0/7	1/7

Table 2. Anticoagulant rodenticide (AR) detections from blood samples collected from wild American Kestrels (*Falco spurvarius*) in northern Utah in 2019–2020. Reported values (ng/mL) represent concentrations averaged across three replicate samples. If no rodenticide was identified or if the observed concentration was less than the method detection limit (DL) it was classified as not detected (ND). Care should be taken when evaluating results below the quantitation limit (QL, as indicated with *), as the variability will be greater than the acceptable method performance. Note that only samples with positive AR detections are shown here, while samples without any AR detections ($n = 73$) are not. M = male; F = female; U = unknown sex.

ID	Age	Sex	No. ARs Detected	Brodifacoum[a] (ng/mL)	Bromadiolone[a] (ng/mL)	Difethialone[a] (ng/mL)
Kestrel 1	adult	M	1	18.8	ND	ND
Kestrel 2	adult	F	2	6.96	0.725	ND
Kestrel 3	adult	U	1	6.95	ND	ND
Kestrel 4	adult	F	2	1.85	9.47	ND
Kestrel 5	juvenile	U	1	1.1*	ND	ND
Kestrel 6	adult	F	3	0.73*	2.75	0.67*
Kestrel 7	adult	M	3	0.57*	0.909	14.1
Kestrel 8	adult	M	1	ND	1.2	ND

[a] DLs and QLs for the compounds are as follows: Brodifacoum, DL = 0.48 ng/mL, QL = 1.60 ng/mL; Bromadiolone, DL = 0.14 ng/mL, QL = 0.466; and Difethialone, DL = 0.44 ng/mL, QL = 1.47 ng/mL.

samples opportunistically collected from dead individuals (representing seven raptor species) within the study area revealed AR exposure in seven of eight raptor species tested. However, there was also high heterogeneity in AR exposure rates among age classes, with only one of 59 juvenile nest samples exhibiting AR exposure. Our findings augment extensive prior research demonstrating that raptors are exposed to ARs on a global scale (e.g., van den Brink et al. 2018). Overall, we detected five ARs in liver samples, with brodifacoum the most prevalent, being detected in over half (14 of 27) of the liver samples. Brodifacoum is the most potent AR registered in the United States, is highly toxic to birds, and is also highly persistent in the liver, which contributes to its bioaccumulation (Rattner and Harvey 2021).

Six of 27 of the liver samples processed, including three of eight of tested kestrel samples, had summed second generation AR concentrations >0.1 μg/g wet weight. Toxicity is likely affected by several factors, making a simple concentration threshold indicative of toxicity problematic (Rattner and Harvey 2021). Robust determinations of AR toxicosis are best derived from residue data in combination with signs of toxicity (e.g., overt hemorrhage, hemorrhage revealed at necropsy and/or by histopathology, and/or coagulopathy); the design and logistics of the present study did not permit an evaluation of potential toxicity. Nonetheless, we believe that the relatively high summed AR concentrations found in kestrels are notable and warrant further investigation as a potential threat to kestrels, especially considering evidence suggesting that AR exposure may be a population-limiting factor for the Eurasian Kestrel in the United Kingdom (Roos et al. 2021).

Results from kestrel blood samples indicated relatively high rates of exposure of adults (more

Table 3. Results of a generalized linear model testing for effects of age, habitat, year, and sampling methodology (i.e., blood pooled from multiple juveniles in the same nest or an individual sample) on AR exposure collected from wild American Kestrels (*Falco sparverius*) in northern Utah in 2019–2020.

Coefficient	Coefficient Estimate	Standard Error	*t*	*P*
(Intercept)	217.232	221.457	0.981	0.330
Age (juvenile)	−0.634	0.114	−5.543	<0.001
Landscape (urban)	−0.018	0.075	−0.240	0.811
Landscape (wildland)	−0.123	0.084	−1.459	0.149
Year	−0.107	0.110	−0.978	0.332
Pooled sample	−0.011	0.070	−0.158	0.875

than half of the sampled individuals were exposed) and low rates of nestling exposure (approximately 2% exposure rate of juvenile nest samples). Pooling samples from juveniles within a nest as we have done here could alter AR detection rates, e.g., by diluting AR concentration if only one of several nestlings was exposed, and this should be considered when comparing exposure rates with adults. Importantly, Murray (2020) showed in Red-tailed Hawks that individuals diagnosed with AR toxicosis had detectable quantities of ARs in both liver and serum, while asymptomatic individuals had detectable AR quantities only in liver. If applicable to kestrels, detection of ARs in kestrel blood in our study could indicate recent exposure and the potential for AR toxicosis in some of the adult kestrels sampled. However, mere exposure to ARs does not constitute a determination of adverse effects or toxicosis and we did not note any obvious signs of toxicosis in sampled individuals. Furthermore, disparate exposure rates between adults and juveniles suggests differential exposure pathways by age class. For example, adults may eat more rodents but deliver more insects to nestlings (Sarasola et al. 2003), which could lead to differential exposure by age. This hypothesis requires further investigation (see below).

Although our findings provide preliminary insights into wild American Kestrel exposure to ARs in western North America, we urge caution in extrapolating from these initial findings and considering biases associated with sample methodology that are present in this and other similar studies (van den Brink et al. 2018). Our sample size of positive exposures from both liver (six of eight kestrels tested positive) and blood (seven of 12 adults positive, one of 59 juvenile nest samples positive) were small. Additionally, we caution that the individuals sampled in this study may not be representative of the wild population of kestrels or of spatial distribution of AR exposure in the region. For example, liver sampling occurred in kestrels found dead in the field or admitted to a rehabilitation facility that were injured, sick, or moribund. Dead or fatally ill kestrels could be disproportionately likely to have been exposed to ARs. Furthermore, blood sampling of adults was opportunistic and kestrels that had an AR exposure history could have been easier to trap for a variety of reasons. Also, some of the ARs (e.g., brodifacoum) can be detectable for months, such that individuals could have been exposed outside of the study area, either on migration or when wintering. Blood sampling of nestlings within nest boxes is likely a more representative sample of the nestling population than blood sampling of adults is of the adult population, although natural nest cavities in the region were more difficult to monitor and sample, which is an important caveat. Lastly, it is expected that rodenticide application varies within the study area and it is recommended that use patterns of ARs be investigated in order to provide context to kestrel exposure patterns.

Research Recommendations. We recommend additional blood sampling of adult kestrels because of the high exposure rates identified in the present study. Our sample size was small, and the study design did not permit evaluation of any relationship between adult exposure and sublethal responses of AR exposure (e.g., breeding success), but with a larger sample size, this would be possible. In future studies, doing a clotting time assay of birds from which blood is drawn could provide additional evidence and context of exposure. We also recommend exploring the development of sampling and analytical techniques to test for ARs in kestrel excreta (Esther et al. 2022), which can easily be collected from nest boxes post-fledging and from captured wild birds. The potential to passively and noninvasively sample wild populations via excreta sampling after juveniles fledge from a nest box represents a potentially valuable technique that could provide insights into exposure throughout the full nesting season. We recommend monitoring adult kestrel foraging ecology and prey deliveries to nests, with a combination of in-person behavioral observations and camera traps installed at nest boxes, to identify prey density, composition, and distribution. Such data would also allow assessment of any differences in diet between adults and nestlings (e.g., Sarasola et al. 2003) that could explain the variable blood exposure rates between age classes, although physiological and metabolic factors should also be considered, as well as variation in spatial AR application rates. Tracking adult kestrels with radio or satellite telemetry could enable quantification of kestrel home ranges, habitat use, and potential AR exposure pathways, both on breeding territories as well as throughout the full annual cycle. We recommend collecting information on AR spatial use, including the application methods and frequency and the types of products being used, which could help researchers interpret results and identify exposure pathways. Finally, we stress the importance of long-term studies of wild raptor populations that move beyond documenting AR

exposure and toward quantifying potential population-level responses.

SUPPLEMENTAL MATERIAL (available online). Table S1: Analytical method accuracy and precision for 13 rodenticides in American Kestrel liver samples. Table S2: Analytical method accuracy and precision for 13 rodenticides in American Kestrel whole blood samples.

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