

Causes of mortality and unintentional poisoning in predatory and scavenging birds in California

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ABSTRACT

Objectives: We documented causes of mortality in an opportunistic sample of golden eagles, turkey vultures and common ravens, and assessed exposure to several contaminants that have been found in carrion and common prey for these species.

Methods: Dead birds were submitted for testing through wildlife rehabilitation centres and a network of wildlife biologists in California from 2007 to 2009.

Results: The leading causes of mortality in this study were collision-related trauma (63 per cent), lead intoxication (17 per cent) and anticoagulant rodenticide poisoning (8 per cent). Elevated liver lead concentration (≥ 2 $\mu\text{g/g}$) and bone lead concentration (>6 $\mu\text{g/g}$) were detected in 25 and 49 per cent of birds tested, respectively. Approximately 84 per cent of birds tested had detectable rodenticide residues. The majority of rodenticide exposure occurred in peri-urban areas, suggesting that retail sale and use of commensal rodent baits, particularly in residential and semi-residential areas in California, may provide a pathway of exposure.

Conclusions: Monitoring anthropogenic causes of mortality in predatory and scavenging bird species provides important data needed to inform on mitigation and regulatory efforts aimed at reducing threats to these populations.

INTRODUCTION

Predatory and scavenging birds, by virtue of their foraging ecology, are exposed to toxicants present in wild and domestic prey. These species are at risk of lead poisoning when they inadvertently ingest lead shot or fragmented bullets in crippled prey and animal carcasses or discarded viscera (Hunt and others 2006, Knopper and others 2006, Pauli and Buskirk 2007). Upon impact, lead bullets can fragment into hundreds of pieces, resulting in contamination of animal carcasses (Hunt and others 2006). Harmful levels of lead exposure and poisoning have been documented in a wide range of predatory and scavenging bird species (Fisher and others 2006, Pain and others 2009), including the endangered California

condor (*Gymnogyps californianus*) (Finkelstein and others 2012, Johnson and others 2014). In response to high lead-related mortality in the reintroduced California condor population, a ban of lead ammunition used for big game and non-game hunting went into effect within the condor range in California in 2008 (California Department of Fish and Game 2008, California State Assembly 2008). In 2013, California broadened these regulations to restrictions on lead ammunition for the taking of all wildlife using a firearm statewide by 2019 (California State Assembly 2013).

Studies have also demonstrated exposure to anticoagulant rodenticides and related mortality in predatory birds and to a lesser extent in scavenging birds (Newton and others 1990, Berny and others 1997, Howald and others 1999, 2009, Stone and others 1999, 2003, Eason and others 2002, Lima and Salmon 2010). Predatory wildlife is exposed to anticoagulant rodenticides through consumption of live prey animals suffering sublethal toxicosis. Scavengers appear likely to become exposed primarily through foraging on animals that have died from rodenticide poisoning. However, some bird species have also been documented to directly consume rodenticide bait (Howald and others 1999, 2009).

Unlike first-generation anticoagulant rodenticides, which have a relatively short half-life (Hadler and Buckle 1992) and require multiple exposures to kill target species, second-generation anticoagulant rodenticides, such as brodifacoum, bromadiolone and difethialone, are extremely persistent and acutely toxic with a lethal dose delivered through a single feeding (Environmental Protection Agency 2004). Brodifacoum poses the greatest overall risk to non-target wildlife and has been linked to mortalities worldwide (Newton and others 1990, James and others 1998, Robertson and Colbourne 2001, Eason and others 2002, Stone and others 2003).



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In 2008, the US Environmental Protection Agency released a risk mitigation decision limiting the sale and distribution of rodenticides by December 2009 (Environmental Protection Agency 2008). Prior to this decision, brodifacoum, bromadiolone and difethialone were registered for use against commensal rodents in residential, industrial, commercial and agricultural buildings and could be purchased by the public (Environmental Protection Agency 2008). However, second-generation anticoagulants in consumer products for household use and above-ground bait stations around agricultural buildings still remain for sale in 'consumer' stores such as grocery and hardware stores as of 2013 (California Department of Pesticide Regulation 2013). In 2013, the California Department of Pesticide Regulation proposed regulatory changes that would restrict the use of second-generation anticoagulants to only certified applicators.

Because lead and anticoagulant rodenticide poisoning threaten some populations of predatory and scavenging birds (Cade 2007, Hernández and Margalida 2008, Pain and others 2009), baseline information regarding exposure and associated morbidity and mortality in these species is critical for developing effective management and conservation plans, and assessing the effectiveness of efforts aimed at mitigation. The goals of this research were to document the causes of mortality in an opportunistic sample of predatory and scavenging birds in California—golden eagles (*Aquila chrysaetos*), turkey vultures (*Cathartes aura*) and common ravens (*Corvus corax*)—and to assess exposure to a range of toxicants, including lead from spent ammunition, anticoagulant rodenticides, organophosphorous (OP) pesticides, strychnine and zinc phosphide found in carrion and live prey species serving as sources of food for these species (Redig and others 1982, Heinrich 1988, Engel and Young 1989, Kirk and Mossman 1998, Boarman and Heinrich 1999, Kochert and others 2002, Fleischli and others 2004, Kelly and others 2005). Because these species differ with regard to their foraging ecology, species-specific differences in toxicant burdens may provide further insight into common pathways of exposure for non-target wildlife.

MATERIALS AND METHODS

From 2007 to 2009, we collected blood samples and carcasses of golden eagles, turkey vultures and common ravens through a network of wildlife biologists and wildlife rehabilitation centres throughout California. Wildlife rehabilitation centres that submitted samples for this study included Lindsay Wildlife Museum in the San Francisco Bay Delta region of California, Ojai Raptor Center in southern coastal California, Pacific Wildlife Care in central California and University of California Veterinary Medical Teaching Hospital/Raptor Center and Wildlife Rehabilitation and Release in north central California. These centres rehabilitate birds for

release back to the wild, and individuals that have a poor prognosis for return to full function are euthanased. Date of accession, location found, reason for admission, age class, clinical presentation, treatments and outcome were recorded for each bird. Among birds admitted to the rehabilitation centres, we included only those individuals that had been held in captivity for <3 weeks.

Causes of mortality

Postmortem examinations were performed to assign primary and contributing causes of mortality. We examined each carcass and determined the age class and sex (Kertuu 1973, Henckel 1981, Bloom and Clark 2002), and recorded the location, presentation and severity of lesions. Radiography was used to screen for trauma and metallic opacities in the gastrointestinal (GI) tract, which were retrieved for heavy metal analysis. Microscopic examination of tissues, including brain, spinal cord, peripheral nerves, bone, muscle, tongue, heart, trachea, lung, liver, kidney, gonads, spleen, adrenals, thyroid/parathyroid glands, oesophagus, ventriculus, proventriculus, pancreas, intestines and bursa of fabricius, was performed by veterinary pathologists. Tissue sections were placed in 10 per cent neutral-buffered formalin, paraffin-embedded, sectioned at 5 µm and stained with H&E for histological examination using light microscopy. Special stains were used as necessary.

Primary causes of mortality were assigned based on case history, clinical diagnosis and postmortem findings in conjunction with diagnostic test results. The primary cause of mortality was identified for each individual as the pathological process judged to most likely have resulted in death. Contributing causes of mortality were documented if pathology was identified that increased the probability of death but was not part of the primary disease process. Both primary and contributing causes of mortality were determined in order to assess for potential interactions between mortality factors.

For euthanased birds, the cause of mortality was determined on the basis of the primary pathological diagnosis. Postmortem examinations were performed at the Veterinary Medical Teaching Hospital, University of California, Davis and California Animal Health and Food Safety (CAHFS) Laboratory, University of California, Davis.

Death was attributed to collision-related trauma in birds presenting with a case history and injuries consistent with impact. Mortality from infectious disease was assigned to cases in which there was pathological evidence of disease associated with isolation or detection of an infectious agent by culture, or PCR or pathogen-specific special stains, respectively. Diagnostic testing included RT-PCR on oropharyngeal swabs for influenza A viruses, RT-PCR on kidney tissue for West Nile virus (WNV), PCR on intestinal contents for *Salmonella* species, fluorescent antibody test for *Chlamydophila psittaci*, and aerobic cultures of the liver and lungs on blood agar and MacConkey plates. Birds with histopathological lesions suggestive of WNV infection were tested for WNV

using RT-PCR performed on brain and kidney. Virus isolation (chick embryo inoculation) was performed on pooled tissue homogenates from birds with lesions suggestive of a viral aetiology. Aspergillosis was diagnosed based on fungal culture or identification of characteristic conidiophores and hyphae associated with lesions on histology using special stains.

Lead poisoning was assigned the primary cause of mortality when pathological lesions indicative of lead poisoning and lead concentrations in blood and/or liver compatible with toxicosis ($>100 \mu\text{g/dL}$ and $>5 \mu\text{g/g}$, respectively) (Franson 1996) were detected at the time of death. When tissues were available, lead concentrations were evaluated in blood, liver and bone (femur) samples. For cases in which metal was retrieved from the GI tract during gross examination, heavy metal analysis was performed to substantiate the postmortem examination findings. Blood lead analyses were performed using graphite furnace atomic absorption spectrophotometry (GFAAS). Liver and bone samples, and metallic objects retrieved from the GI tracts of birds during gross examination were analysed for lead concentrations using inductively coupled argon plasma emission spectrometry (ICP-AES). Liver and bone samples were digested in nitric and hydrochloric acid at 180°C and then diluted with 18Ω water prior to analysis. Positive lead results ($>1 \mu\text{g/g}$) by ICP-AES analysis were confirmed using GFAAS with a detection limit of $0.05 \mu\text{g/g}$. Metallic objects were completely digested as described for liver and bone samples and analysed by ICP-AES without GFAAS confirmation due to the high concentrations of lead detected. Initial digests were diluted as needed to have detected concentrations fall within the method standard curve. Lead concentrations were expressed as microgram/gram on a dry-weight basis. Each batch of samples was analysed with blanks matched to the matrix (i.e. blood, liver or bone). At least 10 per cent of the samples were run in duplicate. Results of duplicates needed to match within 15 per cent of the average value. Standard reference materials for the blood samples (Wisconsin State Laboratory of Hygiene (WSLH), Madison Wisconsin) and liver and bone samples (DOLT and TORT, National Research Council of Canada, Montreal, Quebec, Canada) were also run with each batch. Recoveries of lead from the standard reference materials needed to fall within ± 20 per cent of the certified value (or for the WSLH within ± 20 per cent of the refereed average value determined from the proficiency testing trial).

Anticoagulant rodenticide intoxication was assigned the primary cause of death in cases for which lesions indicative of coagulopathy in the absence of trauma were identified on gross and/or histological examination, in combination with hepatic rodenticide concentrations $>0.2 \mu\text{g/g}$, which is a threshold that has been proposed to represent minimum levels at which lethal effects may be seen in predatory birds (Berny and others 1997, Thomas and others 2011). In cases for which anticoagulant rodenticide intoxication was assigned a contributing cause of death, lesions indicative of

coagulopathy were identified in conjunction with the primary disease process. When liver samples were available, hepatic tissue was analysed for concentrations of brodifacoum, bromadiolone, chlorophacinone, coumachlor, difethialone, diphacinone and warfarin. Analyses were performed using high-performance liquid chromatography mass spectrometry. Briefly, following extraction of 5 g samples of liver with glacial acetic acid (0.5 ml) and homogenisation in 50 ml of 5 per cent ethanol in ethyl acetate and 20 g of sodium sulfate, the sample extract was refined by Florisil solid phase extraction or gel permeation chromatography. Anticoagulant concentrations were quantified by liquid chromatography with fluorescence and diode array UV detection and comparison of analyte response in samples with known standards. Each analytical batch of 10 or fewer samples included a negative and a positive control sample, which were extracted and analysed with the submitted samples. Negative controls consisted of purchased bovine liver tissue. Positive control samples consisted of bovine liver tissue fortified with all of the rodenticides at the minimum laboratory reporting limits concentrations. Minimum laboratory reporting limits were $0.01 \mu\text{g/g}$ for brodifacoum, $0.05 \mu\text{g/g}$ for bromadiolone, warfarin and coumachlor, and $0.25 \mu\text{g/g}$ for chlorophacinone, difethialone and diphacinone. Analyte recoveries in positive control samples were calculated for any rodenticides quantified in associated samples. These recoveries were consistently in the 70–120 per cent range.

We screened for OP pesticides, strychnine and zinc phosphide when samples of GI contents of adequate sample volume were obtained ($n=12$ golden eagles and 13 turkey vultures for OP pesticides, $n=12$ golden eagles and 16 turkey vultures for strychnine and zinc phosphide). GI contents were homogenised with 5 per cent ethanol in ethyl acetate and extracts analysed for 42 different OP pesticides by gas chromatography with flame photometric detection (GC-FPD). Extracts high in lipid content were cleansed prior to analysis by gel permeation chromatography. Samples that were positive for an OP by GC-FPD were reevaluated using gas chromatography-mass spectrometry (GC/MS). The minimum laboratory reporting limit for OP pesticides by GC-FPD and GC/MS was $0.1 \mu\text{g/g}$. GI contents were also analysed for strychnine and zinc phosphide using GC/MS. For strychnine, the pH of the samples was adjusted to 10 and then extracted with 4 ml ethyl acetate. The analysis of zinc phosphide was based on the detection of phosphine gas liberated by the addition of sulfuric acid and extracted by solid-phase microextraction from the sample headspace followed by analysis by GC/MS. Minimum laboratory reporting limits for both procedures were $1 \mu\text{g/g}$. Quality control measures for OP pesticides, zinc phosphide and strychnine analysis were similar to those used for rodenticide analysis with negative controls and positive controls analysed with each set of samples. The control matrix used for analysis of GI contents consisted of commercial canned dog food mixed with dog kibble

and adjusted to a pH of approximately 5. Analysis of brain tissue for decreased acetylcholinesterase levels associated with OP pesticide exposure was not possible in this study because brain tissue was used for histopathological examination. All laboratory analyses were performed at the CAHFS Laboratory, University of California, Davis, California, USA.

DATA ANALYSIS

In order to evaluate the relative importance of different causes of mortality in our sample of predatory and scavenging birds, proportionate mortality was calculated as the percentage of birds with deaths attributed to a specific cause among all cases examined for this study. This measure was estimated for the entire sample and for each species separately. We also calculated the percentages of individuals with anticoagulant rodenticide and elevated lead exposure among birds sampled for this study. Anticoagulant rodenticide exposure was defined based on detectable hepatic residues of one or more anticoagulant rodenticides. Elevated lead exposure was determined by published threshold lead concentrations specific to Falconiformes (Franson 1996). According to these thresholds, hepatic lead concentrations (wet weight) ≥ 2 $\mu\text{g/g}$ and bone lead concentrations >6 $\mu\text{g/g}$ (dry weight) indicate elevated exposure (Pattee and others 1981, Custer and others 1984, Franson 1996).

RESULTS

We evaluated 48 carcasses (21 golden eagles, 23 turkey vultures and 4 common ravens) originating from 13 counties in California. Forty-five of these birds were found alive and admitted to centres. Among these 45 cases, 14 died during the rehabilitation process and 31 were humanely euthanased due to poor prognosis for release. The remaining two golden eagles and one turkey vulture were found dead and were submitted for testing.

PROPORTIONATE MORTALITY

Collision-related trauma was the leading cause of mortality accounting for 63 per cent (30/48) of deaths among birds examined for this study (Table 1). Trauma was the primary cause of death for 67 per cent of golden eagles

(14/21). Five of these mortalities were presumed to be due to wind turbine strike because the eagles were found at the base of wind turbines. Collision-related trauma was also the leading cause of mortality in turkey vultures, representing 57 per cent (13/23) of the cases. Vehicular collision was suspected as the cause of trauma for several of these vultures because they were found by roads. In addition, three common ravens died as a result of traumatic injuries. One of these ravens was found at the base of a wind turbine, and the cause of mortality was therefore presumed to be the result of wind turbine strike.

Lead poisoning was the primary cause of mortality in 17 per cent (8/48) of cases (Table 1). Lead-related mortalities occurred during the winter and early spring months and were found in various areas throughout the state. Death was attributed to lead poisoning in 17 per cent (5/23) of the turkey vultures. The diagnosis was based on pathological lesions in combination with elevated hepatic lead concentrations (11, 15, 26 and 38 $\mu\text{g/g}$) in four vultures and elevated blood lead concentration (170 $\mu\text{g/dl}$) in the fifth case. The two vultures with lower hepatic lead concentrations underwent chelation therapy during rehabilitation, thereby lowering their lead concentrations. A lead-based fragment (760,000 $\mu\text{g/g}$), presumed to be from a bullet, was retrieved from the GI tract of one vulture.

Lead poisoning was the primary cause of death in 14 per cent (3/21) of golden eagles. Diagnoses were made based on pathological changes and elevated hepatic lead concentrations in two cases (28 and 36 $\mu\text{g/g}$) and a high blood lead concentration (314 $\mu\text{g/dl}$) in the third case. Lead and anticoagulant rodenticide intoxication were assigned as contributing causes of mortality for a fourth golden eagle that died from acute trauma due to a vehicular collision. The hepatic concentrations of lead and brodifacoum in this eagle were substantially elevated at 5 and 1.7 $\mu\text{g/g}$, respectively.

Eight per cent (4/48) of the birds in our sample died due to anticoagulant rodenticide intoxication and all were turkey vultures (Table 1). This was the primary cause of mortality in four vultures (17 per cent; 4/23) that had moderate to high hepatic concentrations of brodifacoum (0.28, 0.76, 0.76 and 0.77 $\mu\text{g/g}$). Three of these four cases originated over a two-week period in

TABLE 1: Primary and contributing causes of mortality in 23 turkey vultures, 21 golden eagles and 4 common ravens submitted by wildlife rehabilitation centres and biologists in California from 2007 to 2009

Cause of mortality	Golden eagle		Turkey vulture		Common raven	
	Primary	Contributing	Primary	Contributing	Primary	Contributing
Trauma	14	0	13	0	3	0
Lead intoxication	3	1	5	0	0	0
Anticoagulant rodenticide intoxication	0	1	4	3	0	0
Infectious disease	2	1	0	0	0	0
Burn	2	0	0	0	0	0
Gunshot	0	0	1	0	1	0

late August and were clustered within 5 km of each other in a suburban area of California. Anticoagulant rodenticide intoxication was a contributing cause of mortality in two vultures that died due to collision-related trauma and in one vulture noted above with primary lead intoxication. The hepatic brodifacoum concentrations in these vultures were 0.36 and 0.42 µg/g, and 0.77 µg/g, respectively. Brodifacoum exposure, with hepatic concentrations ranging from 0.11 to 0.18 µg/g, was considered an incidental finding in three vultures and two common ravens that died from other causes (collision-related trauma, lead intoxication and gunshot). In addition, brodifacoum exposure (0.34 µg/g) was documented as an incidental finding in one golden eagle that died from wind turbine strike.

Infectious disease was the primary cause of mortality for two cases in this study. WNV infection was the cause of death for one golden eagle, and systemic aspergillosis was the primary cause of mortality for another eagle. No other diseases or contributing conditions were detected in the individual with aspergillosis. Evidence of viral infection was found on histopathology in two golden eagles and two vultures that died due to trauma. Virus isolation and WNV PCR were negative in these cases. Other primary causes of mortality included electrocution (one golden eagle), gunshot (one vulture and one raven) and fire (one golden eagle).

PREVALENCE AND MAGNITUDE OF EXPOSURE TO TOXICANTS

Elevated liver lead concentration (≥ 2 µg/g) was detected in 21 per cent of the 39 birds tested (Table 2), indicating acute elevated exposure. In addition, among the 37 bone samples tested, 48 per cent had elevated lead concentrations (>6 µg/g), indicating chronic exposure to lead (Table 2). Among birds with detectable lead levels ($n=8$ in liver and $n=30$ in bone), concentrations ranged from 3.4 to 38 µg/g for liver (median=21 µg/g) and 1 to 71 µg/g (median=8 µg/g) for bone.

Anticoagulant rodenticides residues were detected in 84 per cent (32/38) of the 38 birds tested (Table 3). Rodenticide exposure was detected in 100 per cent (4/4) of common ravens, 95 per cent (18/19) of turkey

vultures and 67 per cent (10/15) of golden eagles (Table 3). Among birds with detectable rodenticide residues ($n=32$ for brodifacoum, $n=4$ for bromadiolone and $n=2$ for difethialone), concentrations ranged from 0.01 to 1.7 µg/g (median=0.15 µg/g) for brodifacoum, 0.06 to 0.15 µg/g for bromadiolone (median=0.06 µg/g) and 0.26 to 0.27 µg/g for difethialone. Two golden eagles and two turkey vultures had detectable levels of multiple anticoagulant rodenticides with brodifacoum residues present in all four cases. Furthermore, brodifacoum was the sole anticoagulant detected in the four rodenticide-related mortalities. Other anticoagulant rodenticides that were detected, but not associated with coagulopathy on postmortem examination, were bromadiolone in three golden eagles and one turkey vulture and difethialone in one eagle and one vulture. OP pesticides, strychnine and zinc phosphide were not detected in any of the birds examined for this study.

DISCUSSION

Collision-related trauma was the leading primary cause of death in this study. This finding is consistent with results from research conducted elsewhere in which impact injuries were common causes of morbidity and mortality in predatory birds (Deem and others 1998, Morishita and others 1998, Wendell and others 2002). Most of the collision-related trauma mortalities in vultures in this study were attributed to impacts with motor vehicles. Turkey vultures often scavenge along roads (Kelly and others 2007) and, therefore, may be at higher risk for vehicular collision, relative to other species. Several golden eagles in this study died as a result of wind turbine collisions. These cases originated from the Altamont Pass Wind Resource Area (APWRA), a wind farm located in an area that provides habitat for a high density of golden eagles (Hunt and Hunt 2006). Rough estimates of the number of golden eagles killed by wind turbine blade collisions each year in the APWRA range from 40 to 116 (Hunt and Hunt 2006, Smallwood and Thelander 2008). Routine monitoring is conducted around the turbines and injured golden eagles are transported for treatment to one of the wildlife rehabilitation centres submitting samples for this study (Lindsay Wildlife Museum). Impacts with

TABLE 2: Hepatic and bone lead concentration (µg/g) ranges and percentage of individuals with concentrations exceeding threshold levels for elevated exposure (≥ 2 µg/g for liver and >6 µg/g for bone) in a sample of golden eagles, turkey vultures and common ravens submitted by a network of wildlife rehabilitation centres and biologists in California from 2007 to 2009

Species	Liver				Bone			
	n	Number of ND results	Range µg/g	% of samples ≥ 2 µg/g	n	Number of ND results	Range µg/g	% of samples >6 µg/g
Golden eagle	15	11	(ND-36)	26%	15	5	(ND-59)	46%
Turkey vulture	20	16	(ND-38)	20%	18	2	(ND-71)	50%
Common raven	4	4	NA	0	4	0	(3-26)	50%

Results are expressed in µg/g (dry weight).
ND, not detected.

TABLE 3: Hepatic anticoagulant rodenticide concentration ($\mu\text{g/g}$) ranges and percentages of individuals with detectable anticoagulant rodenticide residues in a sample of golden eagles, turkey vultures and common ravens submitted by a network of wildlife rehabilitation centres and biologists in California from 2007 to 2009

Species	n	Brodifacoum			Bromadiolone			Difethialone		
		Range $\mu\text{g/g}$	Number of ND results	% of samples	Range $\mu\text{g/g}$	Number of ND results	% of samples	Range $\mu\text{g/g}$	Number of ND results	% of samples
Golden eagle	15	(ND-1.70)	5	67%	(ND-0.06)	12	20%	(ND-0.27)	14	7%
Turkey vulture	19	(ND-0.77)	1	95%	(ND-0.15)	18	5%	(ND-0.26)	18	5%
Common raven	4	(0.01-0.18)	0	100%	ND	4	0	ND	4	0

Results are expressed in $\mu\text{g/g}$ (dry weight).
ND, not detected

man-made structures, including collisions with vehicles, are common findings in mortality studies of predatory and scavenging birds (Keran 1981, Morishita and others 1998, Wendell and others 2002, Erritzoe and others 2003, Erickson and others 2005, Harris and Sleeman 2007, Rodriguez and others 2010). The high prevalence of traumatic injuries in these studies reflects the significance of threats posed to predatory and scavenging birds by land-use change associated with development. Because wild birds suffering from trauma related to vehicular collision and collision with man-made structures have a higher probability of detection compared with birds suffering from other causes of disease in remote areas, proportionate mortality estimates for collision-related trauma may be inflated relative to other less easily detected causes of mortality in our study.

Lead and anticoagulant rodenticide poisoning were also common causes of mortality in this study. Jointly, lead and rodenticide toxicity accounted for one-fourth of the mortality and contributed to the deaths of three additional cases for which trauma was the proximate cause of death. Cases of lead poisoning occurred during the winter and early spring months outside of the big game hunting season in California. These findings are consistent with studies of free-ranging California condors, golden eagles and turkey vultures in which lead exposure has been documented throughout the year in California (Pattee and others 1990, Hall and others 2007, Sorenson and Burnett 2007, Kelly and Johnson 2011, Kelly and others 2011) and provide further evidence that year-round shooting activities contribute to lead exposure in these species.

Rodenticide exposure was widespread among birds tested for this study. A number of studies have also documented anticoagulant rodenticide exposure in predatory and scavenging birds throughout North America and Europe (Newton and others 1990, Hosea 2000, Stone and others 2003, Albert and others 2010, Lima and Salmon 2010). These studies present a common theme of increasing frequency of anticoagulant rodenticide exposure, most notably brodifacoum, bromadiolone, difenacoum and difethialone, in predatory and scavenging bird species (Thomas and others 2011). Turkey vultures exhibited the highest magnitude of anticoagulant rodenticide exposure and associated mortality, which may be due, in part, to the relatively higher proportion of carrion in their diet. Residues were also identified in all ravens examined. In addition to exposure through opportunistic scavenging, ravens have also been reported to directly consume rodenticide bait (Howald and others 1999, 2009). Golden eagles had a lower prevalence of anticoagulant rodenticide exposure relative to ravens and vultures, which may reflect a lower risk of exposure for birds with relatively greater live prey in their diet. Similar patterns of exposure based on foraging ecology have been documented in predatory and scavenging birds in Scotland (Hughes and others 2013).

In addition, the lower burden of exposure may also be due, in part, to the preferred habitat for golden eagles, characterised by open undeveloped areas in California (Kochert and others 2002). The majority of exposed birds in our study and other studies conducted in California originated from semi-residential areas and undeveloped lands with urban edges. Anticoagulant rodenticide exposure was documented in 69 per cent of 74 non-target mammals and birds sampled in California, 95 per cent of which came from areas with significant urban development (Hosea 2000). In addition, an assessment of rodenticide exposure in predatory birds in both urban and agricultural areas in five counties in California found anticoagulant rodenticide residues in 92 per cent of the individuals (Lima and Salmon 2010). Because the majority of cases had residues from second-generation anticoagulant rodenticides, which are registered only for the control of commensal rodents in and around structures, Lima and Salmon (2010) suggested that the greatest risk to wildlife is the retail sale and use of commensal rodent baits, mainly residential areas in California.

Several birds in this study had elevated concentrations of lead in bone, reflecting chronic or repeated exposures over the individual's lifetime (Stendell 1980, Anderson and Havera 1985, Scheuhammer and Dickson 1996). Similarly, many individuals had anticoagulant rodenticide residues without evidence of coagulopathy on postmortem examination. The sublethal effects of lead and anticoagulant rodenticide exposure and their role as contributing factors of mortality in wild animals are largely unknown (Walker and others 2008, Hunt and others 2012) and are likely more important than we can determine from studies of free-ranging populations. In many instances, death is the result of a myriad of deleterious health effects, some of which are not evident on postmortem examination. Chronic lead exposure from prolonged or repeated exposure at lower concentrations can have sublethal effects by impairing reproductive success, growth rate of young birds, neurobehavioral function, immunity and physiology (Hunt and others 2012).

Although scientific evidence is lacking for wild birds, it seems possible that the probability of mortality with anticoagulant exposure may increase with traumatic events as exposed individuals are potentially more susceptible to fatal haemorrhage precipitated by minor trauma or exertion (Eason and Murphy 2001). In addition, anticoagulant rodenticide exposure may predispose individuals to death from other stressors, such as starvation and disease (Newton and others 1999, Riley and others 2007, Albert and others 2010, Leemus and others 2011), and can also slow growth in developing chicks (Albert and others 2010). Similarly, lead exposure may impair an individual's behaviour or locomotion, increasing the likelihood of a traumatic event (Mendenhall and Pank 1980, Newton and others 1990, Burger and Gochfeld 2005, Kelly and Kelly 2005).

Because lead and rodenticide poisoning pose a threat to predatory and scavenging birds, monitoring exposure and associated morbidity and mortality in these species is vital for guiding management and conservation planning. Reducing use of lead ammunition and anticoagulant rodenticides in critical habitat for protected species may be an important mitigation effort that could compensate for other major, less preventable, causes of mortality (i.e. collision-related trauma). Although sample selection is biased towards cases more easily detected in settings frequented by people, opportunistic sampling of wildlife presenting to rehabilitation centres offers an efficient and non-invasive means for monitoring morbidity and mortality in wild bird populations (Wendell and others 2002, Rodriguez and others 2010). California has approximately 100 permitted wildlife rehabilitation centres that annually rehabilitate nearly 2000 predatory and scavenging birds (Carion 2012). These centres present valuable opportunities for monitoring spatial and temporal patterns of morbidity and mortality of free-ranging wildlife. Sustained monitoring for lead and anticoagulant rodenticide exposure in predatory and scavenging birds presenting to wildlife rehabilitation centres provides an opportunity for assessing trends and effectiveness of increasing regulatory efforts aimed at reducing the threat of lead and anticoagulant rodenticide-related mortality in vulnerable wildlife in California.

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Data sharing statement Data underlying analyses in this study are available upon request.

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