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Turkey Vultures (*Cathartes aura*) from Southern California are Exposed to Anticoagulant Rodenticides Despite Recent Bans

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ABSTRACT.—Secondary poisoning with anticoagulant rodenticides (ARs) has been identified as an important threat for raptor conservation worldwide. In 2019, the California State Legislature passed Assembly Bill 1788 (made effective in 2020), which prohibits or limits the use of second-generation anticoagulant rodenticides (SGARs) in the state, as a follow-up to the California Department of Pesticide Regulation's ban on SGARS implemented in 2014. Currently, the adherence to these recent restrictions on ARs in southern California is unknown. To assess whether these bans prevented exposure of raptors and other wildlife to ARs, we investigated (1) the prevalence of exposure to eight different ARs in the blood of Turkey Vultures (*Cathartes aura*) before and after the 2019 ban, and (2) the distribution of resighted (encountered) wing-tagged Turkey Vultures included in this study to assess where exposure might occur. Of 27 Turkey Vultures tested for eight ARs, one out of 11 sampled in 2017 had detectable (trace) but not quantifiable levels of difethialone, and two out of 16 (12.5%) sampled in 2021 had detectable levels of diphacinone (one had 8 ppb; another indicated as positive without quantification). Overall, the prevalence of exposure to ARs was 11.1% (3 of 27), 7.4% for diphacinone and 3.7% for difethialone. Based on 93 resightings of 20 of the wing-tagged Turkey Vultures, all but one remained within the areas of Los Angeles, San Bernardino, Orange, Riverside, and San Diego Counties of southern California. Our study suggests that the exposure risk of Turkey Vultures to ARs persisted despite recent restrictions. Our small sample size and reliance on blood in live vultures rather than liver tissue in dead ones may be underestimating true ARs exposure in our study population. We propose a continued and integrated monitoring approach that includes measurements of ARs in both free-ranging (blood samples) and deceased (liver samples) Turkey Vultures for effective large-scale monitoring. This approach will assess compliance with current and future bans and regulations regarding the use of these poisons in California.

KEY WORDS: *ban*; *Cathartidae*; *difethialone*; *diphacinone*; *monitoring*; *prevalence*; *rodenticide*; *scavengers*.

CATHARTES AURA EN EL SUR DE CALIFORNIA ESTÁN EXPUESTOS A RODENTICIDAS ANTICOAGULANTES A PESAR DE RECIENTES PROHIBICIONES

RESUMEN.—La intoxicación secundaria con rodenticidas anticoagulantes (RA) ha sido identificada como una amenaza importante para la conservación de las aves rapaces en todo el mundo. En 2019, la Legislatura del Estado de California aprobó el Proyecto de Ley 1788 de la Asamblea Legislativa, efectivo en el 2020, que prohíbe o limita el uso de rodenticidas anticoagulantes (RA) de segunda generación (RASG) en el estado,

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como seguimiento a su prohibición, implementada en 2014, por parte del Departamento de Regulación de Pesticidas de California. Actualmente se desconoce el cumplimiento de estas recientes restricciones sobre los RA en el sur de California. Para evaluar si estas prohibiciones evitaban la exposición a estos RA en aves rapaces y animales silvestres, investigamos (1) la prevalencia de exposición a ocho RA en la sangre de *Cathartes aura* antes y después de la prohibición de 2019 y (2) la distribución de los encuentros visuales de *C. aura* marcados con bandas alares incluidos en este estudio para evaluar dónde podría producirse la exposición. De los 27 *C. aura* analizados para ocho RA, uno de 11 *C. aura* muestreados en 2017 tuvo niveles detectables (trazas) pero no cuantificables de difetialona, mientras que dos de los 16 (12.5%) *C. aura* muestreados en 2021 tenían niveles detectables de difacinona (uno tenía 8 ppb; otro indicado como positivo sin cuantificación). En general, la prevalencia de exposición a los RA fue del 11.1% (3 de 27), del 7.4% para la difacinona y del 3.7% para la difetialona. Sobre la base de 93 encuentros visuales de los 20 *C. aura* marcados todos, menos uno, permanecieron en los condados de Los Ángeles, San Bernardino, Orange, Riverside, y San Diego en el sur de California. Nuestro estudio sugiere que el riesgo de exposición de los *C. aura* a los RA persistió a pesar de las recientes restricciones. Aunque se basa en un tamaño de muestra pequeño y con las limitaciones de analizar únicamente la sangre en aves vivas en lugar de los hígados de aves muertas, nuestro estudio sugiere que el riesgo de exposición de *C. aura* a los RA persistió incluso después de que se implementaran las recientes restricciones. Proponemos un enfoque de seguimiento continuo e integrado que debe incluir la investigación de RA tanto en *C. aura* de vida libre utilizando muestras de sangre como en individuos muertos por medio de muestras hepáticas para un seguimiento efectivo a gran escala y para evaluar el cumplimiento de las prohibiciones y regulaciones actuales y futuras con respecto al uso de estos venenos en California.

[Traducción de los autores]

INTRODUCTION

Secondary poisoning with anticoagulant rodenticides (ARs) has been identified as an important threat for raptor conservation worldwide (Rattner et al. 2014, Elliott et al. 2016, Gómez et al. 2022). These compounds interfere with the synthesis of vitamin K-dependent coagulation factors in the liver of raptors and other animals who ingest them through the prey or carrion they feed upon (Hindmarch and Elliott 2018, Nakayama et al. 2019, Oliva-Vidal et al. 2022). Depending on the type, amount, and frequency of AR ingestion, raptors may show variable degrees of coagulopathy, hemorrhage, and blood loss, and eventually die as result of circulatory collapse and hypovolemic shock (Murray 2017, 2018, 2020).

As a measure to reduce the impact of ARs on raptors and other wildlife species, the California State Legislature passed the California Ecosystems Protection Act of 2019 (Assembly Bill 1788; entered into effect in 2020), prohibiting or limiting the use of second-generation ARs (SGARs) in the state (Quinn et al. 2019), and as a follow up to the California Department of Pesticide Regulation's ban on SGARS implemented in 2014. Currently, in California, products containing SGARs (e.g., brodifacoum, bromadiolone, difenacoum, and difethialone) can be purchased and used only by certified pest control companies and operators under very specific circumstances and are no longer sold or approved for consumer use. Furthermore, the California State Legislature recently passed a moratorium on diphacinone (Assembly Bill 1322), a first-generation AR (FGAR) still available to consumers. The ban became effective in January 2024. The effectiveness of these regulations and their enforcement remain unknown and will certainly depend on effective enforcement and political will. Despite this legislation, the use of rodent baits with ARs appears to be a persistent and common practice in natural, urban, and suburban areas of California (e.g., Kelly et al. 2014, Gabriel et al. 2018). This causes concern about the population impact on sensitive southern California raptors, including those now considered extirpated in certain areas, such as the breeding Burrowing Owl (*Athene cunicularia*; Bloom 2023), which is known to be affected by ARs in Arizona (Justice-Allen et al. 2017) or the White-tailed Kite (*Elanus leucurus*), a small mammal specialist (Dunk 2020) that is currently suffering from unexplained population declines in our study area (P. Bloom unpubl. data).

Several studies have reported variable prevalence of exposure of California raptors to ARs in the past (Lima and Salmon 2010, Kelly et al. 2014, Krueger et al. 2015, Franklin et al. 2018, Gabriel et al. 2018). Recently, scavengers, like the critically endangered California Condor (*Gymnogyps californianus*) and the non-threatened Turkey Vulture (*Cathartes aura*) have also had a high prevalence of exposure to ARs (Herring et al. 2022, 2023). These findings indicate ARs as a persistent, pernicious threat for raptors that may contribute an additive mortality factor for raptor populations (Roos et al. 2021).

Raptors have proven reliable indicators of environmental toxicological risk (Redig and Arent 2008, Gómez-Ramírez et al. 2014), thus serving as sentinel

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species. Continued monitoring of the use of ARs in natural, rural, urban, and suburban areas, and studies aimed at quantifying the likelihood of secondary poisoning of raptors, are needed to assess the effectiveness of recent regulations and help reduce threats to raptors and other wildlife (Quin et al. 2019). These studies are usually based on the identification and quantification of ARs in the livers of raptors that have been admitted to rehabilitation centers and subsequently died or were euthanized because of their injuries or medical conditions (Slankard et al. 2019, Gómez et al. 2023, Elliott et al. 2022). Mortalities and other specimens from rehabilitation centers can provide a robust number of samples to assess environmental prevalence of ARs. Nevertheless, estimates of AR prevalence from rehabilitated and dead birds may be biased, as reliance on birds admitted to rehabilitation centers overestimates prevalence of exposure and dose received (Gómez et al. 2022).

Studies of the prevalence of AR exposure in free-ranging raptor populations are rare. This has been recently accomplished using liver samples from culled Barred Owls (*Strix varia*) and Barred/Spotted Owl hybrids in the western USA, as part of a program aimed to reduce the impact of these birds on the Spotted Owl (*Strix occidentalis*; Gabriel et al. 2018, Hofstadter et al. 2021). Another approach has been the use of whole blood (or plasma/serum) for AR testing, yielding variable prevalence of exposure (Kwasnoski et al. 2019, Herring et al. 2022, Oliva-Vidal et al. 2022). The nonlethal, random sampling of free-ranging birds also avoids the killing of animals for investigating AR exposure, which is particularly valuable in regards of animal welfare and in declining and/or endangered species. Blood collection also enables the repeated sampling of recaptured birds. Unfortunately, a major caveat of this approach is an apparent lower sensitivity of testing blood compared with liver samples (Murray 2020, Herring et al. 2022, Oliva-Vidal et al. 2022). This may be a result of the short half-lives of ARs in blood, which only indicates recent exposure, from days to weeks (Murray 2020, Herring et al. 2022, Gómez et al. 2022); conversely, liver samples usually indicate chronic, longer-term AR exposure, persistence, and bioaccumulation (Gómez et al. 2022). Recently, Herring et al. (2022) compared liver and blood AR values in California Condors and Turkey Vultures and found that the prevalence of ARs in blood was much lower (10%) than in the liver (93%) of Turkey Vultures. More studies are needed to better understand the pharmacokinetics and toxicokinetics of ARs in non-target animals, and the value of blood, liver, and other tissues for surveillance, as they clearly differ among species and for the specific compound (Horak et al. 2018). Nevertheless, the detection of ARs in blood confirms the presence of these compounds in the environment and sheds light on the recency of exposure in natural populations (Oliva-Vidal et al. 2022).

The Turkey Vulture is an obligate scavenger, feeding on the carcasses of a wide variety of dead animals commonly found in urban, suburban, and natural areas (Kirk and Mossman 2020). Many coastal southern California Turkey Vultures are resident birds (Garrett and Dunn 1981, P. Bloom unpubl. data). As obligate scavengers, Turkey Vultures exploit multiple types of carrion, including dead rodents and domestic and wild carnivores such as bobcats, foxes, coyotes, and weasels, making them susceptible to exposure and bioaccumulation of many environmental poisons and pollutants (Kirk and Mossman 2020). Throughout their extended home range and varied landscapes where they find food, Turkey Vultures can be easily captured and in relatively large numbers (Bloom et al. 2019). Due to their broad distribution, resident status and extensive home range, Turkey Vultures may be useful avian sentinels for ARs, lead, and other pollutants available over large areas (Kelly et al. 2014, M. Saggese unpubl. data), allowing us to assess compliance to the recent state restrictions on the use of ARs and the risk of AR exposure to raptors.

During the past 7 yr, Turkey Vultures from southwestern California, the largest urban and suburban area in the state, were live captured, tagged and released during a collaborative research program aimed at assessing their potential as environmental sentinels for the presence of spent lead ammunition, characterizing their breeding ecology and movements, investigating their exposure to pathogens, and evaluating their population genetics (P. Bloom, M. Saggese, A. Bonisoli-Alquati, A. Koedel, and A. Eagleton unpubl. data). Several studies have reported exposure of Turkey Vultures to ARs (Kelly et al. 2014, Herring et al. 2022, 2023) in California. Our objective in this study was to investigate the prevalence of exposure to eight different ARs in Turkey Vultures from southern California. We hypothesized that the recent state bans on the use of ARs would reduce exposure to ARs in birds captured after the ban (2014 for FGARS and 2019 for SGARs) compared to before the ban. We also investigated the distribution of the Turkey Vultures included in this study to assess where exposure might occur.

METHODS

We trapped Turkey Vultures using a walk-in trap, as previously described (Bloom et al. 2007, 2019) at

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Anaheim Lake (33.867116°N, 117.851124°W), Orange County, southern California, USA, between 2016 and 2021. Once trapped, birds were physically examined, measured, aged, sampled, wing-tagged, and weighed. We accessed Turkey Vulture nests (all nests located in Orange County, P. Bloom unpubl. data) and wing-tagged and sampled five nestlings. Blood (<1% of body weight) was collected from the basilic vein with heparinized syringes. Blood was kept refrigerated until arrival to the laboratory, where plasma was separated by centrifugation at 2500 G × 10 min and saved in cryovials kept at −80°C.

Plasma samples (1.2 mL) from 27 Turkey Vultures were shipped overnight to the California Animal Health and Food Safety Laboratories (CAHFSL; Davis, CA, USA) for AR testing and quantification by liquid chromatography-tandem mass spectrometry for four FGARs (chlorophacinone, warfarin, coumachlor, and diphacinone), and four SGARs (brodifacoum, bromadiolone, difethialone, difenacoum). Quality control samples included both unfortified and fortified bovine calf serum (Sigma-Aldrich). Two fortified serum samples were included at 2.5 ppb and 25 ppb levels of ARs. The lower concentration of 2.5 ppb was used for the reporting limits of all of the reported ARs with the exception of difethialone, which had a reporting limit of 25 ppb. An internal standard, d4-diphacinone, was included with all samples including quality control, and it was verified present for each analysis. We reported an AR as "trace" if detected, but not quantified (when an AR was identified at a concentration below the reporting limit). To test for a difference in prevalence of ARs before and after the 2019 ban, we used Fisher's exact test applied to detection of any of the four SGARs.

We also assessed the movements of the patagial-tagged Turkey Vultures, largely considered resident in the area (southwestern California, west of the Mojave Desert), by mapping all sightings reported to the Bird Banding Laboratory (US Geological Survey, Maryland, USA; retrieved October 2023) to assess and infer where these birds forage and may become exposed to rodenticides. Most observation records included exact encounter coordinates or provided the name of the location (i.e., a city park or nature preserve); in those cases we used approximate coordinates based on the descriptive details provided by the observer.

RESULTS

Of 27 Turkey Vultures tested for eight different ARs, 11 before and 16 after the 2019 bill came into effect, the overall prevalence of exposure to ARs was 11.1% (3 of 27). The overall prevalence of exposure to difethialone was 3.7% (one of 27), whereas the prevalence of exposure to diphacinone was 7.4% (two out of 27). For the 16 Turkey Vultures sampled in 2021, the prevalence of exposure to diphacinone was 12.5% (two out of 16).

Three out of 11 and two out of 16 Turkey Vultures, were nestlings; the remaining birds were all >6 mo old. We did not detect ARs in any of the nestlings. Among the non-nestlings, only one Turkey Vulture sampled in 2017 had detectable (trace) but not quantifiable levels of difethialone, the only SGAR detected. Two Turkey Vultures sampled in 2021 had detectable levels of diphacinone, an FGAR (one had 8 ppb; another indicated positive without laboratory quantification). The prevalence of exposure to the four SGARs among non-nestlings before (one out of 8) and after the ban (zero out of 14) did not differ significantly (odds ratio = 0.00, 95% CI = [0.00, 22.29], $P = 0.364$; Adjusted Cramer's V = 0.19, 95% CI = [0.00, 0.69]).

Twenty of the 27 Turkey Vultures we tagged and sampled were encountered (a total of 93 sightings) between November 2017 and May 2023. Except for one outlier (not shown but observed in San Jose, Santa Clara County), all the marked Turkey Vultures for which we have encounter data (19 out of 27 birds) remained and foraged within the scope of five different counties (Los Angeles, San Bernardino, Orange, Riverside, and San Diego) in southern California (Fig. 1).

DISCUSSION

Results of this study indicate that at least three out of 27 Turkey Vultures (or 11%) were exposed to FGARs and SGARs in a large area of southern California. Although the sample size was small and a larger sample size may have better detected exposure, our study suggests that the exposure risk of Turkey Vultures to ARs persisted after the recent bans were implemented. Such risk may extend to other raptor species. This was not surprising, given that considerable quantities remain in homes for private use and as of this date may still be available on store shelves (P. Bloom unpub. data, M. Saggese unpubl. data) and online, with potential unauthorized use in different urban, suburban, and rural settings. Furthermore, there are still legal exemptions to the recent bans (e.g., agricultural use).

Prevalence of exposure to ARs was generally low, with only one FGAR (diphacinone) and one SGAR (difethialone) detected. Overall, it was also in line with a recent estimate of 10% prevalence of AR exposure in Turkey Vultures' blood (Herring et al. 2022).

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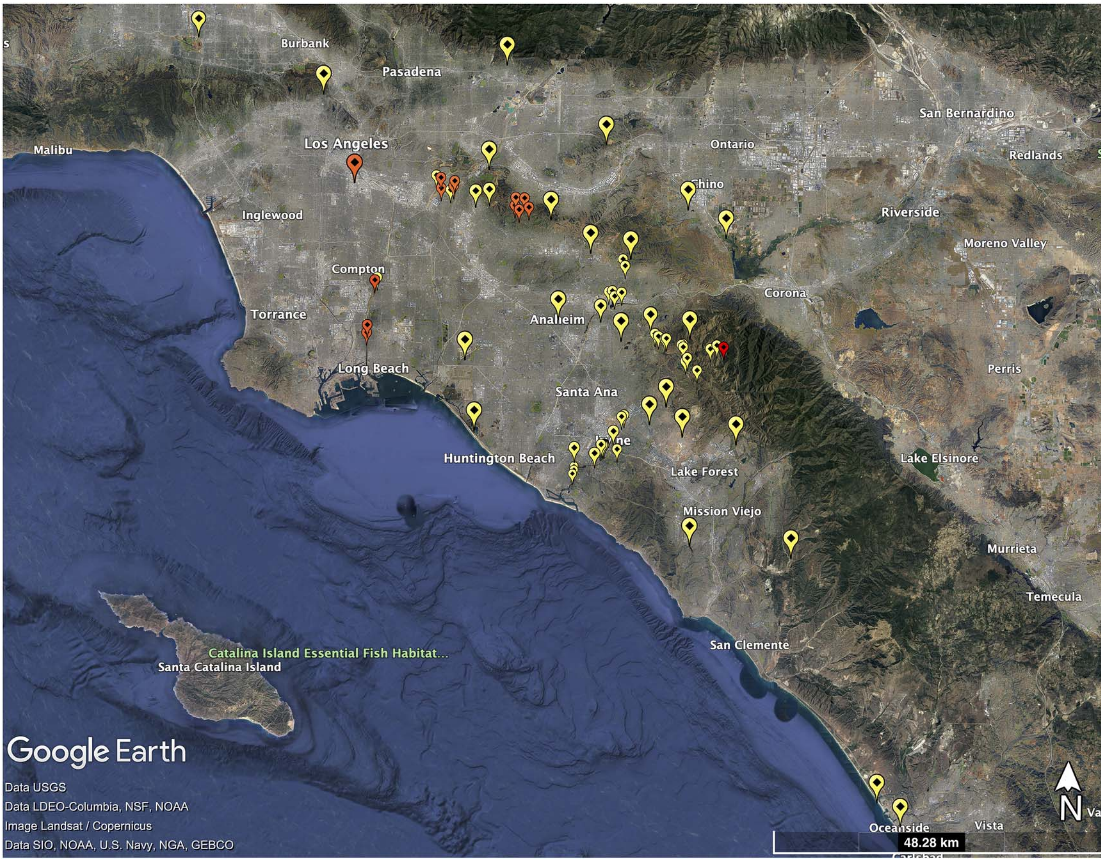

Figure 1. Visual sightings for wing-tagged southern California Turkey Vultures. Each icon in the figure corresponds to a sighting location of one of 19 different Turkey Vultures. Each icon indicates one sighting, except for a few areas in which sighting locations were identical. Larger icons indicate a clearly distinguishable sighting, and smaller icons indicate multiple sightings that were close to each other. Yellow icons indicate birds that were negative for ARs and red icons indicate those that tested positive.

The presence of ARs is of concern, especially for difethialone, an SGAR that since 2014 has been restricted to professional pest control agents and county agencies, for both indoor and outdoor use in California (Elliott et al. 2016, California State Legislature Bill AB 1788). Since 2020, its use has only been allowed under specific circumstances, with the goal of reducing the risk of exposure for nontarget wildlife (Riley et al. 2007, Moriarty et al. 2012, Cypher et al. 2014, Benson et al. 2019). However, we note that the one SGAR detection in our study occurred prior to the 2019 ban.

The other AR detected, diphacinone, is an FGAR offered for public use and under fewer restrictions than SGARs. FGARs are still available for professional use in California for different types of rodent control, and they require multiple exposures to kill rodents. However, the frequent detection of diphacinone in non-targeted wildlife and in baits has resulted in the recent passage of a bill in California placing new restrictions on the use of diphacinone starting on 1 January 2024 (California State Legislature Assembly Bill 1322). Continued monitoring of prevalence of AR exposure in Turkey Vultures may help monitor the efficacy of this ban in reducing environmental concentrations of diphacinone.

We acknowledge that the small sample size of 27 birds analyzed in this study implies that our estimates of prevalence of exposure to ARs should be interpreted with caution. Nonetheless, our results based on blood samples may underestimate the prevalence of exposure to ARs in Turkey Vultures from southern

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California. ARs have a limited half-life in blood, and their detection in blood can only indicate recent exposure (Gómez et al. 2022). Kelly et al. (2014) found a 95% prevalence of exposure to ARs in the liver of 19 Turkey Vultures submitted from different rehabilitation centers in California. However, studies comparing the prevalence of AR exposure in blood and liver are few. For example, in central California, Herring et al. (2022) found a several fold higher prevalence of exposure to ARs in the liver than in the blood of Turkey Vultures. Assuming a similar relationship and based on the high prevalence values found in previous studies, we cannot rule out higher prevalence of exposure in the southern California Turkey Vulture population.

Our goal was to assess whether Turkey Vultures continue to be exposed to ARs after the recent bans, which they do. Although the extent of this exposure is probably higher than the 12.5% we report here for birds sampled after these bans, the ability to sample free-ranging birds of prey suggests that live-captured Turkey Vultures could be useful to assess recent exposure to ARs in a wide environmental range where a pathway of exposure to these highly toxic compounds occurs. We suspect that Turkey Vultures are exposed to ARs by ingesting the liver and potentially the gastrointestinal tract of scavenged animals (Hindmarch and Elliott 2018, Nakayama et al. 2019, Oliva-Vidal et al. 2022).

As changes in California's legislation regarding the use of ARs continue and existing California Legislature bills are enforced, it will be important to monitor the effectiveness and adherence of both the public and professional pest control companies. Raptors are one of the groups more widely studied for the purpose of contaminant surveillance. However, some raptor species have relatively limited home ranges and a large-scale evaluation of AR use in a particular region such as southern California may not be possible by sampling individuals on a broad spatial and temporal scale. However, Turkey Vultures are widely distributed, cover large foraging areas, and can be trapped relatively easily at multiple locations. Their large size allows adequate volumes of blood to be collected, and the broad spectrum of carrion consumed exposes them to multiple prey species potentially contaminated with ARs. These aspects make Turkey Vultures good sentinels for AR exposure and toxic effects in raptors and the environment in general.

The ecological and toxicological significance of the AR levels in blood of Turkey Vultures, as for many other raptors, have not yet been fully determined. The use of blood for evaluating exposure to ARs in scavengers has shown variable, sometimes contrasting, results (Herring et al. 2022, Oliva-Vidal et al. 2022). Further studies comparing blood and liver AR concentrations (paired samples) in birds that die or are euthanized at rehabilitation centers may prove useful to better understand these reported differences through comparative testing. Additionally, using liver tissue from recently deceased vultures to test for ARs will better elucidate the occurrence and intensity of bioaccumulation. The use of blood clotting assays has been recommended (Hindmarch et al. 2019) and could constitute another useful and complementary approach to assess AR exposure and effects in Turkey Vultures. Thus, identifying, refining, and validating methodologies for future studies on AR exposure in this species is key to implementing a monitoring program that will be reliable, effective, and inexpensive. Meanwhile, we propose an integrated monitoring approach that should include both free-ranging and deceased Turkey Vultures for effective large-scale monitoring of AR in southern California.

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