Jonathan Evans

Please find the attached comments and references submitted on behalf of the Center for Biological Diversity and Raptors Are The Solution.

November 7, 2025

Submitted via Smart Comment: https://cdpr.commentinput.com/?id=JsSRaG6NA

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RE: Draft Proposed Anticoagulant Rodenticide Regulations

Deputy Director Teerlink:

These comments are submitted on behalf of the Center for Biological Diversity and Raptors Are The Solution on the Draft Proposed Anticoagulant Rodenticide ("AR") Regulations presented during the September 24, 2025 California Department of Pesticide Regulation ("DPR") Anticoagulant Rodenticide Mitigation Informal Public Workshop.

We appreciate the information and context provided by DPR during the presentation. We are gravely disappointed, however, that instead of strengthening restrictions on these dangerous, bioaccumulative poisons that have infiltrated California's ecosystems, DPR is choosing to weaken the restrictions, which will result in more animals becoming sick or dying from exposure. Instead of weakening the restrictions and attempting to circumvent the intent of the Poison Free Wildlife Act of 2025, and preceding AR-focused laws, DPR needs to narrow the existing loopholes in the law, including that of agricultural use.

The legislature set a higher standard for DPR in adopting regulations related to ARs than any other pesticide under DPR's regulatory control. Importantly, DPR must ensure that any regulations reduce exposure in wildlife and require the implementation of Integrated Pest Management ("IPM") or Sustainable Pest Management ("SPM") before any anticoagulant rodenticide use. Unfortunately, the current draft regulations do not achieve

these critical objectives included in the Poison Free Wildlife Act of 2025, and preceding laws restricting anticoagulant rodenticides.

As DPR is well aware, there are a range of sustainable pest management strategies that do not require the use of ARs for rodent control that can be adopted in a cost-effective manner to successfully address rodent infestations.

Anticoagulant Rodenticide Regulations Must Ensure a Trend of Reduced Wildlife Exposure

The use of anticoagulant rodenticides results in pervasive exposure in non-target wildlife. Despite previous restrictions, the exposure of non-target wildlife to ARs remains high. The most recent publicly available statewide report from the California Department of Fish and Wildlife found that 71.9% of wildlife tested had been exposed to ARs, with exposure to Second Generation Anticoagulant Rodenticides ("SGARs") remaining high. (CDFW 2024). As CDFW noted, "exposures detected in 2023 were most likely related to use after AB1788 was implemented (January 1, 2021)", which means that SGAR exposure rates remain high despite the current moratorium (CDFW 2024). Previous attempts to restrict AR exposure by classifying SGARs as restricted materials have also proven to be ineffective in reducing exposure. (DPR 2018). This is likely due to the ability of ARs to bioaccumulate in non-target wildlife *regardless of the method of use*, indicating that increased restrictions are necessary to avoid continued harm to wildlife.

The Poison Free Wildlife Act and preceding anticoagulant rodenticide laws create a higher burden on DPR in developing restrictions to avoid adverse effects than other pesticides. In order for the current moratorium to be lifted, DPR must make a finding of reduced exposure in wildlife. Specifically, DPR must have "adopted any additional restrictions necessary to ensure a trend of statistically significant reductions in the percentage of wildlife exposed…" (Cal. Food & Agric. Code § 12978.7). DPR's proposed regulations would allow for large expansions of the current exemptions to enabling more use of ARs in restaurants, grocery stores, parks, and wildlife habitat areas, and continue the exemption for agriculture.

Expanding loopholes to allow use in more areas will increase exposure, contrary to the purpose of the law to limit exposure. Estimates project there are between over 86,000-98,000 restaurants in California (National Restaurant Association 2025, Snappy 2025). There are approximately 30,000 grocery stores in the state (Xmap 2024). The California Grocers Association alone represents more than 6,000 brick-and-mortar stores, and approximately 150 grocery supply companies (California Grocers Association 2025). DPR's "Crosswalk of Anticoagulant Legislation and Proposed Regulations" also highlights the expansion to allow First Generation Anticoagulant Rodenticides ("FGARs")

in Wildlife Habitat Areas, and non-production agricultural sites such as cemeteries, golf courses, parks, highways, and railroads "away from manmade structures with the 35 consecutive day duration limit if allowed on the product label" totaling up to 105 days per year (DPR Crosswalk 2025). The proposed ability to use ARs in wildlife habitat areas is shocking and runs contrary to the intent of laws restricting ARs that have consecutively tried to prohibit use in Wildlife Habitat Areas. Expanding use to hundreds of thousands of additional locations, and linear miles of highways and railroads, while continuing the current exempted uses, runs counter to the substantial evidence that there is continued high level exposure even with the restrictive moratorium.

DPR noted that it is important for the regulations to reduce repeat AR exposure for non-target wildlife, reduce the overall amount of ARs in the environment, and reduce how long they are available in the environment (DPR Presentation 2025, slide 4 of 19). DPR proposes some time limitations on certain ARs, such as allowing 105 days of use per year for not longer than 35 consecutive day periods (DPR Draft Regulations 2025). These time limitations allow use up to three times for over a quarter of the calendar year, creating a pathway for repeated exposure in wildlife. DPR provides no evidence or background explaining how a 35 day use period up to three times per year totaling 105 days would reduce exposure, or why that period of time is needed for the efficacy of the ARs.

While these calendar restrictions are proposed for some uses, for others, such as the use of FGARs in agriculture or in water supply and hydroelectric energy, there are no calendar use restrictions, allowing for use throughout the entire year. DPR's proposal to expand uses to over 100,000 new sites at restaurants and grocery stores would increase repeat AR exposure, increase the overall amount of ARs in the environment, and increase how long ARs are in the environment, contrary to the stated intention and the requirement that DPR ensure reduced exposure rates.

DPR's proposed regulations allow for increased use by unlicensed individuals. Despite the legislative requirements to make ARs restricted use materials, DPR has proposed to exempt individuals from the permit requirements for sales, purchase, and use (3 Cal. Code Regs. § 6414). This would allow for greater potential for individuals to misuse materials because they can be purchased and used without the more stringent licensing and oversight requirements typically required for restricted use materials. Misuse of rodenticides by non-licensed professionals has been documented as a means to increase exposure in wildlife (Bartos 2012). Creating a permitting loophole that makes it easier for non-licensed professionals to access and potentially misuse products increasing the potential for exposure in wildlife as well as in children, pets, and other animals.

DPR's proposed regulations would expand usage without evidence of how such expansion would ensure there is reduced exposure when the regulations are enacted, and as the

regulations are implemented throughout the years. DPR must use the "best available science" to demonstrate that there would be reduced exposure (Cal. Food & Agric. Code § 12978.7). DPR's proposed package of regulations have provided no scientific evidence regarding ongoing AR exposure rates or how increasing the allowable use sites would "ensure a trend of statistically significant reductions in the percentage of wildlife exposed" to ARs (Cal. Food & Agric. Code § 12978.7). DPR cannot simply enact regulations without a finding that the regulations result in a reduction of wildlife exposure. DPR's regulations must create a continuing mechanism to "ensure" an ongoing trend of statistically significant reductions in the percentage of wildlife exposed in the future. DPR's monitoring and ongoing obligations to ensure reductions continues into the future.

DPR Must Disclose Findings and Data Regarding AR Effects

Good government requires transparency and public exposure, and the laws restricting ARs detail additional requirements for DPR's AR regulations. In any lifting of the AR moratorium, DPR's findings must be rigorous, including an "analysis regarding exposure pathways, sublethal effects, species sensitivity, and the cumulative and synergistic effects of exposure to anticoagulant rodenticides, including lethal and sublethal effects on wildlife, including rare, sensitive, special status, threatened, or endangered species" (Cal. Food & Agric. Code § 12978.7, emphasis added). Wildlife protected under the federal and state Endangered Species Acts, such as mountain lions, Pacific fishers, San Joaquin kit foxes, and northern spotted owls, have suffered from high rates of AR exposure. DPR must analyze the lethal and sublethal effects of their proposed regulations on these imperiled species that are sensitive to AR exposure.

Any regulations issued by DPR must be accompanied by "concurrence with the Department of Fish and Wildlife [CDFW]." (Cal. Food & Agric. Code § 12978.7) DPR has not described whether CDFW has provided any concurrence on the proposed regulations before they were released. As you know, CDFW has not publicly released the most recent Pesticide Exposures & Mortalities in Non-target Wildlife for the 2024 calendar year. Information on 2024 exposures provides a necessary data point regarding any proposed regulations. AB1322, which restricted diphacinone use, went into effect in 2024, and CDFW data regarding diphacinone exposure after the moratorium is some of the best available science regarding trends for AR exposure, which DPR must analyze. The most recent exposure data from public agencies should be available to the public itself to analyze as part of DPR's regulatory decisionmaking.

DPR Must Strengthen Restrictions Because Exposure Remains High

Evidence continues to demonstrate that AR exposure remains high even with the current legislative moratorium restricting use. The California Dept. of Fish & Wildlife's 2023

Annual Report, “Pesticide Exposures & Mortalities in Non-target Wildlife,” reiterated that “despite the implementation of AB1788 that restricted SGAR-use, non-target wildlife was still at risk of exposure and toxicosis” (CDFW 2024). AR exposure was detected in 71.9% of non-target wildlife tested (CDFW 2024). High rates of exposure continue in many species after the legislative moratorium went into effect, including mountain lions, bobcats, coyotes, foxes, owls, eagles, and hawks (CDFW 2023, CDFW 2024). Exposure rates remain high for California mountain lions at 92.8% (CDFW 2024). The Southern California and Central Coast populations of the mountain lion are protected under the California Endangered Species Act. AR exposure continues to threaten the endangered San Joaquin kit foxes (CDFW 2024).

This incredibly high level of exposure in predatory mammals indicates widespread food web contamination and is far too high to allow for relaxing any of the standards of the moratorium now in place. In fact, the moratorium does not go far enough; anticoagulants should be banned for all users in California except to address public emergencies.

Widespread exposure has also been demonstrated in other studies. Current data from investigations into the exposure to anticoagulants in barred owls in Northern California demonstrate continued exposure to anticoagulant rodenticides throughout that landscape. Specifically, SGARs are still being detected in barred owls, varying in age (1-10+years) throughout the Northern California landscape. Specifically, SGAR makes up the majority of exposures, 36% of over 700 barred owls collected and tested from 2018-2024 (Gabriel 2025).

As many as 12.5 percent of turkey vultures in the Los Angeles area tested positive for anticoagulants in a recent study published in the Journal of Raptor Research (Saggese 2024). Because these birds are obligate scavengers, this exposure demonstrates widespread contamination of the food web. According to study author Miguel D. Saggese, an avian and wildlife researcher at Western University of Health Sciences in Pomona, California, the results “provide further evidence that there is still a problem out there for non-target species” (High Country News 2025).

Another study of turkey vultures and endangered California condors found SGARs in all condor flocks tested: liver AR residues were detected in 42% of the condors (27 of 65) and 93% of the turkey vultures (66 of 71). There was evidence of prolonged blood clotting time in 16% of the free-flying condors. According to the study’s authors, “Exposure to ARs may complicate recovery efforts of condor populations within their current range and in the soon to be established northern California experimental population” (Herring 2022).

Anticoagulant rodenticides have emerged as an important threat in forests of the western United States, including for forest dwelling owls. Sixty-two percent of owl specimens (72 barred and 7 barred and spotted owl hybrids) were exposed to anticoagulant rodenticides,

in particular to SGARs. Females and owls sampled close to the wildland–urban interface were more likely to be exposed to anticoagulant rodenticides. The high rate of anticoagulant rodenticide exposure in barred owls and hybrids provides mounting evidence of an additional risk to state and federally-listed threatened Northern spotted owls (Hofstadter 2021).

Not all anticoagulant poison victims end up being necropsied by CDFW or recorded in studies, so it is likely the data undercounts these animals. For instance, in early October a weak and lethargic turkey vulture was admitted to WildCare, a wildlife rehabilitator in Marin County. (Wildcare 2025). The turkey vulture vomited up blue stomach contents, a possible sign of anticoagulant poisoning. Diphacinone has also been found in wild pigs, demonstrating exposure to wildlife and hunting families (CDFW 2025). These incidents point to the fact that there is more exposure than what the state is recording.

It is important to recognize that the effects of ARs manifest in lethal, sublethal, and cumulative impacts. As CDFW states in its recent report on rodenticide exposure:

> It is important to note that exposure in the absence of toxicosis should not be ignored. The uncertainties about the magnitude and drivers of chronic exposure and/or sub-lethal levels of rodenticide exposure demonstrate the need for continued monitoring. Exposure to ARs may predispose wildlife to excessive hemorrhage following an otherwise non-lethal traumatic injury or increase sensitivity to additional exposure(s).
>
> Additionally, it is important to note that the concentration of ARs quantified in tissue samples does not necessarily equate to risk of toxicosis, as even trace levels (quantities detected below the reporting limit) can be associated with signs of coagulopathy and a toxicosis diagnosis (CDFW 2024).

Many studies emphasize the sublethal and cumulative impacts (CDPR 2018). Only a few of the more recent studies are mentioned here; however please see white paper attached. (RATS Fact Sheet). A 2023 study by Vyas, et al. found that sublethal chlorophacinone exposure can directly or indirectly evoke adverse effects in wild raptors, including the ability to thermoregulate (Vyas 2023). A 2020 study by Rattner, et al. found that exposure to one anticoagulant can cause increased risk when an animal is exposed to additional anticoagulants (Rattner 2020). Anticoagulants can reduce reproductive success in barn owls and reduce body weight and growth of nestlings (Naim 2010, Naim 2011).

Sublethal bromadiolone exposure reduces the body weight and condition of common kestrel nestlings, which can impact fitness and survivability (Martinez-Padilla 2017). Anticoagulant rodenticide exposure to bobcats was measured in two areas in southern California over a 16-year period, revealing high levels of exposure, and association with

disease (Serieys 2015). ARs pose a substantial threat to bobcats, and likely other mammalian and avian predators, living at the urban-wildland interface.

The effects and trends in the aquatic food web from anticoagulants must be analyzed prior to adopting any proposed regulations. A 2024 study by Regenery, et al. found that second-generation anticoagulant rodenticides accumulated in wild fish and were transferred to piscivorous predators via the aquatic food chain (Regnery 2024). This study builds on previous studies of the aquatic food web in Germany and Pennsylvania finding frequently detected residues of anticoagulant rodenticides in primarily piscivorous mammalian predators, despite strictly regulated sale, supply, and use of rodenticides (Facka 2024). Another new study found that Brodifacoum caused coagulopathy, anemia, and mortality in rainbow trout at environmentally relevant hepatic concentrations, indicating "the risks associated with the use of AR for wild fish" and reinforcing the need to prevent emissions at their source (i.e., urban rat baiting campaigns near sewers and waterways) (Schmieg, et al. 2025).

Rodenticide Use Poses Human Health Risks

ARs also pose an unreasonable adverse effect on human health. The most recent data from the National Poison Data System affirms that an unreasonable level of annual poisonings continue: over 2,800 poisonings occurred in 2023 with over 75% of those rodenticide poisonings occurring in children (Gummin 2023). Additionally, evidence continues to mount regarding increased mortality because of exposure to rodenticides. A recent study found "reduced survival among children with [leukemia] previously exposed to rodenticides" (Desai 2025). Rodenticides can lead to other counterproductive outcomes for public health. Studies show that rodenticides can increase disease prevalence in rodents by weakening their immune systems and disrupting their social structures (Murray 2021).

IPM/SPM Must be Implemented Before Any AR Use

DPR must ensure that sustainable pest management is implemented before the use of any anticoagulants. DPR's restrictions on anticoagulant rodenticides "shall include a requirement to *implement* sustainable pest management and integrated pest management practices, such as biological control, habitat manipulation, and modification of cultural practices, *before*" the use of anticoagulant rodenticides (Cal. Food & Agric. Code § 12978.7 (emphasis added)). The development of plans and recordkeeping requirements are important, but, currently, there are no clear requirements in the proposed regulations that those plans be *implemented* before using anticoagulants. Indeed, "implement" isn't even used in the regulations even though that is the language required by the legislature.

The draft regulations include a vague provision that individuals "using anticoagulant rodenticides must follow relevant components of the General Rodent Management Plan when making decisions to apply anticoagulant rodenticides" (3 Cal. Code Regs. § 6414(b)(4)). However, that gives discretion as to what portions of the plan are relevant components to apply, and does not include the implementation of strategies the legislature specifically enumerated: "biological control, habitat manipulation, and modification of cultural practices" (Cal. Food & Agric. Code § 12978.7). For example, a user could decide that the only relevant component is "maintaining records," "toxicity scales," "product rotation," or "pest management threshold," which would eliminate the action forcing steps to implement "biological control, habitat manipulation, and modification of cultural practices."

We encourage DPR, at a minimum, to include a requirement that applicators certify that IPM/SPM methods of "biological control, habitat manipulation, and modification of cultural practices" have been implemented before any ARs are used.

DPR's regulations must provide for a mechanism of oversight and enforcement to ensure that IPM/SPM is implemented before any ARs are used. DPR must ensure that there is ongoing implementation of IPM/SPM before any repeated use of ARs, and not simply before the first use.

Alternatives to Rodenticides

There are a wide range of cost-effective alternatives available today. For example, California has over 100 EPA registered non-anticoagulant rodenticide alternatives to anticoagulant rodenticides. This range of registered rodenticide alternatives doesn't even take into account the range of methods to reduce rodent infestation through mechanical, physical, and biological methods. Sustainable and cost-effective rodent control begins with exclusion and sanitation, which are integral parts of any rodent pest management system. Rodent fertility control has increasingly become a viable solution to reducing rodent populations without harming non-target species (Siers 2020, RATS 2023). Without holistic rodent management, rodenticides are an inadequate, short term, perpetually expensive, and counterproductive solution. Resources for sustainable alternatives are readily available via online resources such as SafeRodentControl.org or RaptorsAreTheSolution.org/Got-Rats.

Rodent fertility control has increasingly become a viable solution to reducing rodent populations without harming non-target species. The benefits of a contraceptive based approach may extend to lowered intraspecific competition and lowered disease burden associated with high-density populations. The idea behind rodent contraception is simple, yet grounded in ecology: when population density is lower, disease transmission drops and rodent conflict (which can also spread disease) declines. Paired with proven strategies like

improved sanitation (such as removing food waste and adequate trash collection) and rodent-proofing our homes and storage facilities, contraceptives offer a science-based, humane, and effective rodent control method.

ContraPest is a contraceptive registered in California that uses a combination of 4-vinylcyclohexene diepoxide and triptolide. Laboratory evaluations of ContraPest demonstrated highly effective suppression of reproduction in wild-caught black rats (Siers 2020). In Seattle, a mixed-use business district pilot study managed by Raptors Are The Solution in 2022 illustrated cost savings and effective rat population management using ContraPest as a replacement for anticoagulants, reducing the rat population by 91 percent in just three months (RATS 2023). The maker of ContraPest, Senestech, has also demonstrated the viability of another contraceptive product, Evolve, in a nine-month trial at UC Irvine's residential community, where declining product consumption over time indicated a reduced rodent population (Senestech 2025). At Olsen's Grain and Mill in Chino Valley, AZ, rodent numbers were reduced by 98 percent over 17 months with a 95 percent reduction in product losses showing successful application of rodent fertility control (Good Bites) in an agricultural production facility (Mayer 2025).

Alternatives to Rodenticides Are Working

Since the more stringent restrictions on anticoagulants went into effect in California, data suggests alternative rodent control methods have proven effective without anticoagulant rodenticides. For example, data obtained from seven major county public health/vector control/environmental health departments through Public Records Act requests indicates that rodent complaint numbers since the first anticoagulant bill was implemented do not show significant increases in annual complaints. (Figure 1)

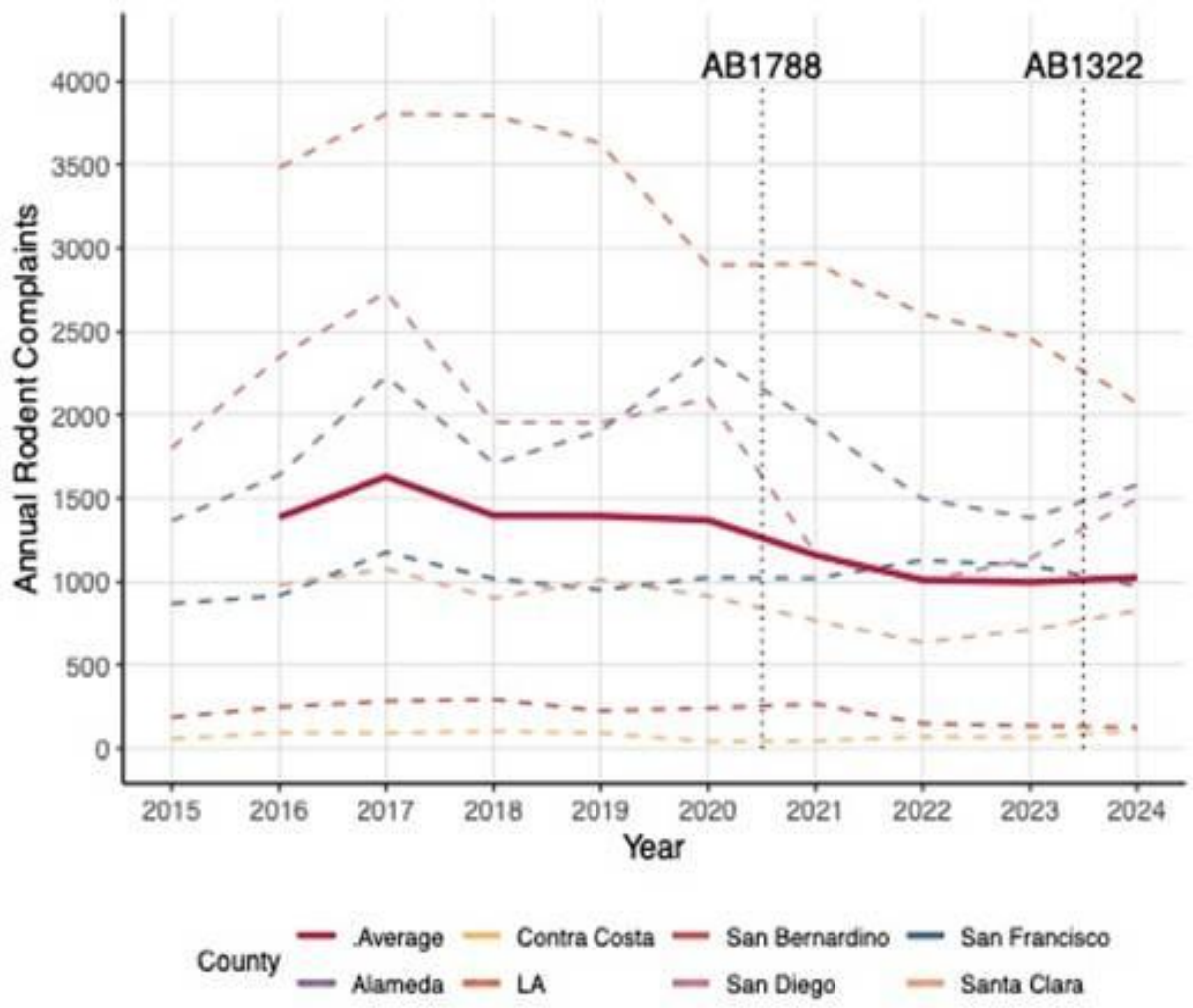

Figure 1- Rodent complaints to public health/vector control/environmental health departments for seven major California counties after rodenticide restrictions.

Conclusion

We remind DPR that it is obligated to follow the intent and requirements of the suite of laws passed restricting rodenticide use in California. The Poison Free Wildlife Act requires DPR to follow the best available science in adopting regulations and it cannot ignore the wealth of evidence demonstrating an ongoing trend of high levels of exposure in wildlife. To date, DPR has provided no scientific evidence that its proposed regulations would reduce exposure in non-target wildlife. Unfortunately, the draft proposed mitigations appear to circumvent the letter and intent of the law and in a premature step, weaken the existing moratorium rather than strengthen protections for wildlife as required.

We urge DPR to ban the use of ARs except for public health and environmental emergencies.

Sincerely,

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Use of Anticoagulant Rodenticides in Single-Family Neighborhoods Along an Urban-Wildland Interface in California

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Urbanization poses many threats for many wildlife species. In addition to habitat loss and fragmentation, non-target wildlife species are vulnerable to poisoning by rodenticides, especially acutely toxic second generation anticoagulant rodenticides (SGARs). Although such poisonings are well documented for birds and mammals worldwide, the pathways by which these widely available compounds reach non-target wildlife have not been adequately studied, particularly in urban landscapes. Long-term studies of wild carnivores in and around Santa Monica Mountains National Recreation Area, a national park north of Los Angeles, have documented >85% exposure to anticoagulant rodenticides among bobcats, coyotes, and mountain lions. To investigate potential mechanisms of transfer of chemicals from residential users of rodenticides to non-target wildlife in the Santa Monica Mountains in Los Angeles County, California, we distributed surveys to residents in two study areas on the north (San Fernando Valley) and south (Bel Air-Hollywood Hills) slopes of these mountains. We assessed knowledge of residents about the environmental effects of rodenticides, and for information about individual application of chemicals. We asked for the same information from pest control operators (PCOs) in both study areas. Forty residents completed the survey in the San Fernando Valley area, and 20 residents completed the survey in Bel Air-Hollywood Hills. Despite the small number of total responses, we documented a number of important findings. Homeowners (as opposed to gardeners or PCOs) were the primary applicators of rodenticides, predominantly SGARs, and awareness of the hazards of secondary poisoning to wildlife was not consistent. Some residents reported improperly applying rodenticides (e.g., exceeding prescribed distances from structures), and in one instance a respondent reported observing dead animals outside after placing poison inside a structure. Improper application of SGARs that ignores label guidelines occurs in neighborhoods along the urban–wildland interface, thereby providing a transmission pathway for chemical rodenticides to reach native wildlife. Moreover, the responses suggest that even on-label use (e.g. placing poisons inside) can create risk for non-target wildlife.

Keywords
Anticoagulant, non-target species, urban carnivores, secondary poisoning, second generation anticoagulant rodenticides

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INTRODUCTION

Rodent control is a widespread activity in the U.S. Of the $90 million per year that residents spend on rodent control products, 90% of those products are in the dry bait category, such as anticoagulants (U.S. Environmental Protection Agency 2006). Genetic resistance to the first-generation anticoagulant rodenticides (e.g., warfarin) has led to development of a second generation of anticoagulant pesticides that are used against small mammal pests of households and agricultural crops (i.e., Norway and black rats, *Rattus norvegicus* and *R. rattus*, and house mice, *Mus musculus*) (Hadler and Buckle 1992). Second-generation anticoagulant rodenticides (SGARs; e.g., brodifacoum, bromadiolone, difethialone, difenacoum, and flocoumafen) are faster acting, more toxic, and more persistent in the environment than their first generation predecessors (Hadler and Buckle 1992; Whisson 1996). Although successful at controlling rodent pests, SGARs globally also contribute to non-target species mortality, such as in New Zealand (Alterio 1996), France (Lambert et al. 2007; Berny and Gaillet 2008), Britain (McDonald et al. 1998; Shore et al. 2003), and Canada (Thomas et al. 2011). In the US, many non-target species have been poisoned by SGARs (Stone et al. 1999; Way et al. 2006; Riley et al. 2007; Uzal et al. 2007; U.S. Environmental Protection Agency 2008; Albert et al. 2010).

Rodents that ingest SGARs may display behaviors that facilitate the ability of predators to capture them (Cox and Smith 1990). Internal hemorrhage greatly affects limb movement, thereby increasing lethargy and decreasing mobility of poisoned rodents. Cerebral hemorrhages can interrupt thigmotaxis, a behavioral mechanism that would normally lead an animal to maximize use of available cover (Cox and Smith 1990; Brakes and Smith 2005). Therefore, we might expect poisoned rodents to be at greater risk of being captured as prey than healthy animals. In turn, opportunistic predators may be at a particular risk because they seek prey that can be caught easily. Consumption of either prey or carcasses contaminated with rodenticides may lead to poisoning of a predator (Brakes and Smith 2005; Rattner et al. 2011). SGARs can even affect wildlife as a result of consuming contaminated invertebrates, contaminated soil, or baits that have been removed from bait stations by rodents (Dowding et al. 2010). Even if products are used inside buildings, poisoned rodents may travel outside where predators could catch them (Stone et al. 1999).

Non-target species that have been documented as being exposed to SGARs in the United States and Canada include barn owl, barred owl, and great horned owl (Albert et al. 2010), gray squirrel, raccoon, white-tailed deer, and red-tailed hawk (Stone et al. 1999), bobcat, coyote and mountain lion (Way et al. 2006; Riley et al. 2007; Uzal et al. 2007), and red fox, striped skunk, and raccoon (U.S. Environmental Protection Agency 2008). In New York State during a 27-year period brodifacoum was involved in 84% of the poisoning cases evaluated (Stone et al. 1999). In one instance, the source of the exposure was determined to be brodifacoum applied in barns and sheds where an owl subsequently was found nearly dead from exsanguination caused by a small laceration on a toe (Stone et al. 1999). This example documents that even though rodenticides were used inside buildings, poisoned rodents traveled outside where predators could catch them. Secondary poisoning — where a non-target species consumes a poisoned target species — caused by these compounds has also been linked to increased disease prevalence, specifically increased susceptibility to parasitic mange in bobcats (Riley et al. 2007).

Urban carnivores are predisposed to secondary poisoning because of habitat use in proximity to residential neighborhoods where these poisons are used (Riley et al. 2003; Gehrt and Riley 2010). In fact, besides road kills, poisoning by rodenticides has been identified as a cause of mortality for urban coyote (*Canis latrans*; Gehrt and Riley 2010), bobcat (*Lynx rufus*; Riley et al. 2010), San Joaquin kit fox (*Vulpes macrotis*; Cypher 2010), and mountain lion (*Puma concolor*; Beier et al. 2010). Others suspect that SGARs may be used to intentionally poison wildlife (Way et al. 2006). The prevalence and severe consequences of SGAR intoxication warrant further investigation.

Use of rodenticides in the agricultural conditions in Europe has been investigated through user surveys (Tosh et al. 2011). These results indicated that users were generally aware of the effects on non-target species, but did not always follow all best practices for application (Tosh et al. 2011). In contrast, few residential users in a previous study in California were aware of non-target species impacts (Morzillo and Mertig 2011a). The application practices of residential users on the urban–wildland interface are not well described, which motivated this study.

We investigated rodent control in a region where secondary poisoning of carnivores has occurred (Riley et al. 2007; Gehrt and Riley 2010). Our objective was to determine potential starting points of pathways through which rodenticides applied at single-family residences eventually could reach non-target wildlife. In other words, we asked, where might anticoagulant rodenticides enter the "natural" environment? Besides describing rodenticide use, we sought to confirm that one SGAR pathway to non-target species is through improper applications by homeowners. SGAR label instructions specify that the baits be applied "inside and along the outside walls of buildings" (U.S. Environmental Protection Agency 1998). We also assessed user knowledge of non-target impacts and compared use of rodent control methods by residents with those of licensed Pest Control Operators (PCOs).

METHODS

This research was a senior-level student-directed project as part of the Environmental Science Practicum at the University of California, Los Angeles (UCLA). There, seniors pursue research projects for an off-campus client, in this instance, the National Park Service at Santa Monica Mountains National Recreation Area (SMMNRA). For purposes of student training, the class was separated into two groups, each with its own study area adjacent to SMMNRA.

Study Areas

Each study area represents an area of urban–wildland interface where residential neighborhoods overlap with habitat of native wildlife, including mountain lions, bobcats, and coyotes. Extensive exposure to anticoagulant rodenticides has been reported within and surrounding SMMNRA (Riley et al. 2003; Riley et al. 2007; Gehrt and Riley 2010). Morzillo and Mertig (2011a, b) evaluated factors affecting use of chemical rodenticides by homeowners in an area adjacent to the western boundary of the current study area.

San Fernando Valley (SFV). This study area contained low- to medium-density residential development, as well as some commercial development and golf courses (Figure 1).

The 101 and 405 Freeways border the study area on the north and east. We further defined the northern boundary of the study area as Ventura Boulevard because it marks the northern (inland) extent of the Santa Monica Mountains.

Bel Air-Hollywood Hills (BA-HH). This study area included the coastal slope of the Santa Monica Mountains south of the 405 Freeway and the 101 Freeway intersection (Figure 1). This area is characterized by highly fragmented open space interspersed with residential development in canyons (Beverly Glen, Benedict, Coldwater, Laurel) and on ridgelines (e.g., Bel Air, Beverly Hills, and Hollywood Hills). Open space lies to the west and Griffith Park (largest natural park in the city of Los Angeles; 1,744 ha) is found to the east. This area is almost exclusively low-density residential with many large homes.

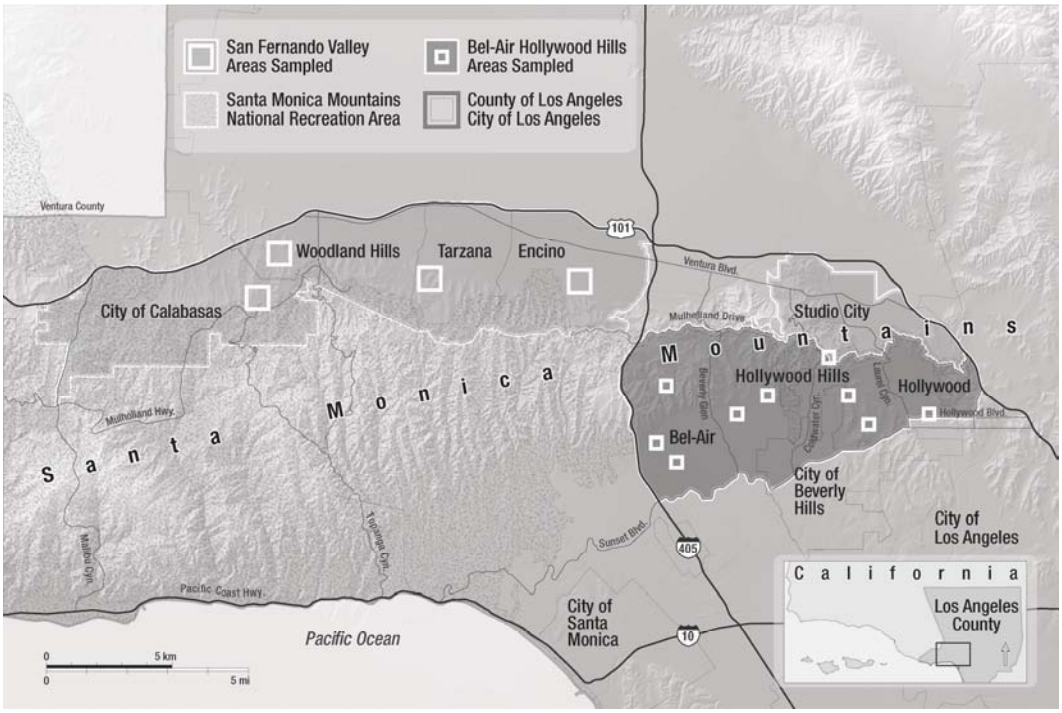

Figure 1. Study areas in San Fernando Valley and Bel-Air to Hollywood Hills. Fliers were distributed to residences indicated by squares.

Survey Design

We developed a series of questions to collect information about rodenticide use, application, and knowledge about related environmental effects (see Appendix A). We employed our survey using an online questionnaire. This method was used because of its low-cost advantage, as well as ease of accessibility, delivery, and response times (e.g., Couper 2009; Poole and Loomis 2009). We acknowledge that several concerns, such as coverage error and potential for response inconsistencies have been linked to use of internet questionnaires (e.g., Couper 2009; Poole and Loomis 2009).

The first part of the survey included an introduction to inform participants of the purpose of the survey, consent information, a description about how the data would be used, and an estimate of the time it would take to complete the survey (Warwick and Lininger 1975). The next several sections investigated if rodenticides were used, products used, target species, application process, and awareness of non-target effects. To ensure recall of the type of rodenticide used, we provided a list of brand names with photographs. Respondents therefore had both the names of the products and a visual reminder of the color and design of the packaging to make their choices about use of chemical rodenticides. We also asked general demographic questions including income, property size, education, age, and ethnicity. All questions in the survey except date of birth were closed questions. Each question was contained on its own webpage to avoid confusion. Finally, the survey ended with a "thank you" for the participants and an invitation to enter into a random drawing for a $50 gift card. The UCLA Institutional Review Board granted the use of human subjects (IRB Exempt Protocol #10-065).

Recruitment of Participants

In March 2010, we contacted Home Owners Associations (HOAs) and Residents Associations for assistance with recruiting resident participants for the online survey. In SFV, two associations agreed to participate; one announced the study using a digital flier, and the other in a digital newsletter. For associations where no residents responded to the electronic solicitation, we also distributed fliers door-to-door (see Appendix B). All recruited participants were limited to occupants of single-family residences.

We placed fliers either on the door handle or on the doormat, with the UCLA seal and title of the project clearly visible. When homeowners were present, we briefly explained the project and invited them to participate. Fliers were placed near the gate or the security keypad of gated properties.

In SFV, we focused on the areas closest to SMMNRA (Riley et al. 2006). This area included areas within Encino, Woodland Hills, Calabasas, and Tarzana. For each of the areas, we randomly selected grids from the Thomas Guide Map, 2007 Edition; each grid contained 250–350 homes. In BA-HH, we used Google Earth to create a quarter-mile-square grid within this study area. We used a random number generator to select nine grid cells within BA-HH (Figure 1). If a selected area lacked residential areas, we used the random number generator to select replacement areas until we had 9 suitable areas. We then walked door-to-door and distributed fliers. In SFV, we delivered 1,200 fliers. In BA-HH we delivered 460 fliers. The difference in the number of fliers is attributed to variation in building density.

Pest Control Operator Interviews

We interviewed managers of pest control operators (PCO) to obtain information about the types of chemicals used, techniques used to apply chemicals, distribution of these chemicals (i.e., where and when they were used), as well as the primary reasons that homeowners retained their services (see Appendix C). We used a phone directory to compile a list of PCOs for each study area and randomly selected companies to sample. We also initiated contacts to any PCO reported by respondents to the online survey.

RESULTS

Survey of Residents

In SFV, 53 people completed online survey; 13 of these responses did not qualify for further analysis. In BA-HH, we received response from 21 residents; one of these responses did not qualify for further analysis. The age of respondents between the two areas did not differ (Student's T test, $p < 0.80$; average age = 55) nor did their ethnicity (Chi-square, $p < 0.27$; overall 95.5% white) or education level (Chi-square, $p < 0.83$; overall 87.9% with bachelor's degree or more).

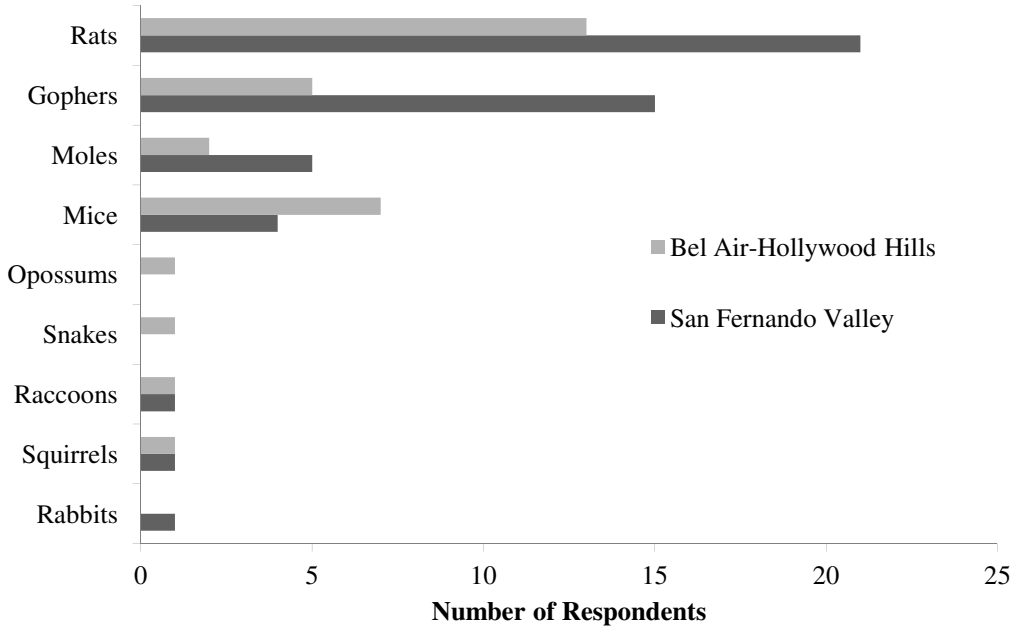

Figure 2. Target species for homeowner rodent control for two study areas in urban–wildland interface areas of the Santa Monica Mountains, Los Angeles County. Respondents could select more than one target species. Several responses were volunteered (raccoons, snakes and rabbits).

In SFV, 65% of respondents used some form of rodent control on their property within the last year, as did 75% in BA-HH. Rats were the most commonly cited target species in both locations, followed by mice and gophers in BA-HH, and gophers and moles in the SFV (Figure 2). Despite the greater proportion of respondents targeting gophers in SFV, the profile of target species was not significantly different between the two areas (Pearson's Chi-square, $p < 0.37$).

Most households applied rodent control themselves in both SFV (62.5%, 25 of 40) and BA-HH (60%, 9 of 15). Gardeners also applied rodent controls (SFV = 17.5%; BA-HH = 6.6%). In BA-HH area, 28% of respondents hired a pest control company but also applied chemicals themselves.

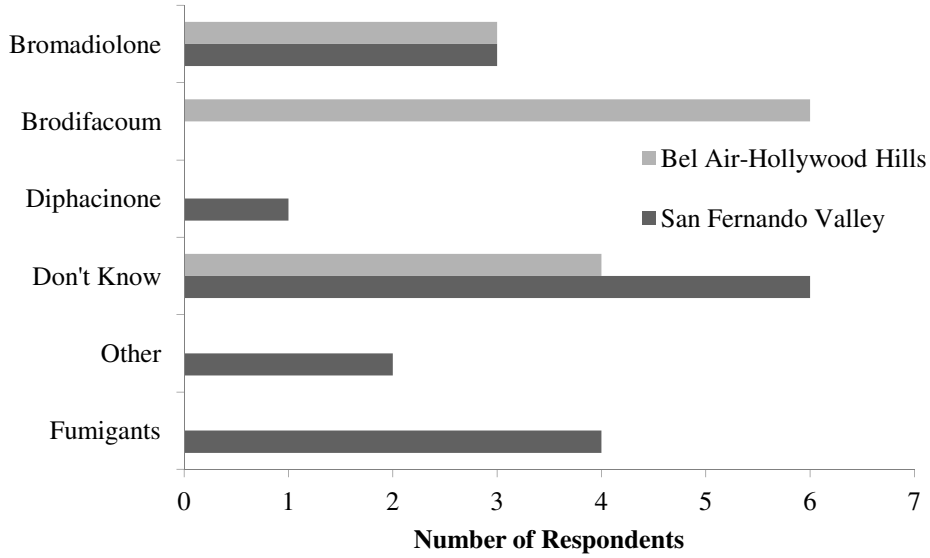

Figure 3. Types of chemical rodenticide used on residential properties in two study areas in urban–wildland interface areas of the Santa Monica Mountains, Los Angeles County. Respondents were able to select multiple answers. Active ingredients from brand name products are listed.

The most commonly reported chemicals in SFV were fumigants, whereas SGARs (active ingredient brodifacoum) were most common in BA-HH (Figure 3). For both areas together, respondents who used anticoagulant rodenticides either could not recall a specific brand name, or if they did, 12 of 13 products uses reported were second-generation (i.e., brodifacoum or bromadiolone). The profile of rodenticides used in the two areas differed substantially (Pearson's Chi-square, $p < 0.09$), with the fumigants being used in SFV and not in BA-HH.

In both locations, households that indicated use of anticoagulants, respondent application of it ranged from monthly to twice per year or variably. From the categories provided on the survey, 10 SFV and 5 BA-HH respondents reported placing SGARs outside away from walls up to 300 and 100 feet away from buildings respectively (Figure 4). Homeowners observed dead rodents (target species) outside after chemical application in both study areas. The median distance category was 1–10 feet for both SFV and BA-HH, and ranged upwards to 30–100 feet away. Of the respondents who placed SGARs outdoors, four observed dead animals outdoors. One homeowner placed a product only *inside* his garage and subsequently found dead animals both inside and outside of the structure.

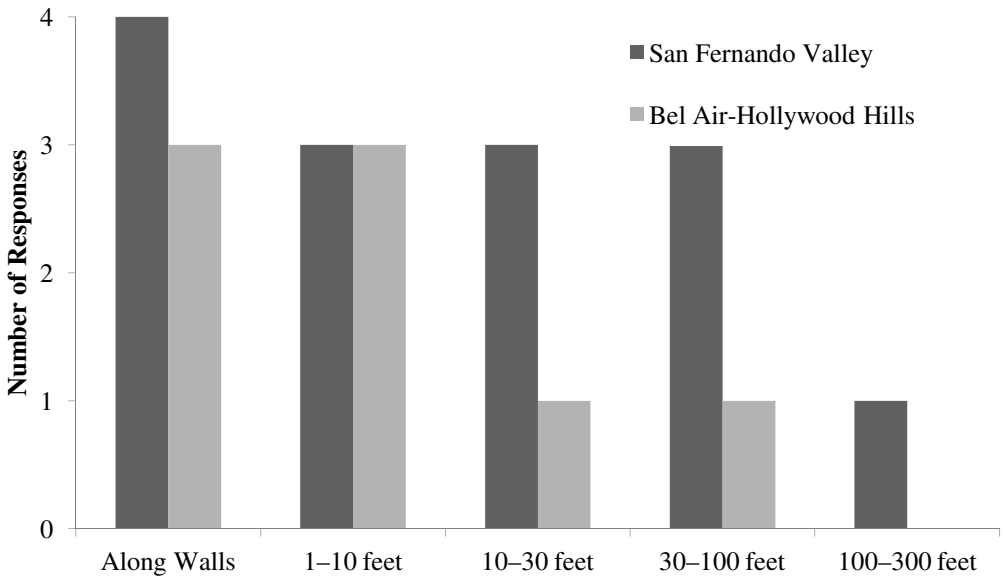

Figure 4. Distribution of anticoagulant rodenticide application outdoors on residential properties in two study areas in urban–wildland interface areas of the Santa Monica Mountains, Los Angeles County. Respondents were able to select multiple answers.

In SFV, 66% of participants (4 of 6) admitted knowing that chemicals used in rodent control, as well as anticoagulants, might be affecting local wildlife. In BA-HH, 35% homeowners (7 of 20) knew about effects of SGARs on wildlife. Five people did not know and 8 people did not answer the question.

Surveys of Pest Control Operators (PCOs)

Five of 23 PCOs contacted in SFV responded to our survey. All 5 PCOs stated that they primarily control mice and rats, and use snap traps. Four also responded that they use chemical baits, and 2 used exclusion techniques. For those that used chemicals, 3 used SGARs and 2 used available first generation anticoagulants.

All PCOs stated that the main reason they are contacted is because of indoor rodents; two of those PCOs also stated as many calls about rodents in outdoor landscaping. All 5 companies inform homeowners about products used; 2 companies inform homeowners about locations of traps or bait. All PCOs reported placing rodenticides within 1 foot of fences and buildings, while one each reported placement up to 60 feet from buildings.

Only 2 of 37 (5.4%) PCOs from the BA-HH area responded. Neither company used chemicals; both used snap traps and exclusion techniques.

DISCUSSION

Homeowners reported applying rodenticides in ways that are prohibited by package instructions. Thus, this is a probable pathway for transfer of SGARs to other wildlife. Because our study areas

are known to have nearby carnivore populations, we can speculate that wildlife may encounter the poison directly, and, more importantly, can encounter as contaminated prey animals, alive or dead.

The two compounds (brodifacoum, bromadiolone) most frequently detected by Riley et al. (2007) in mammalian carnivores were the same most frequently reported as used by respondents in our survey (Figure 3). Similarly, bromadiolone and brodifacoum were the two most common compounds found in more than 100 mountain lions tested from around the state of California (R. H. Poppenga, personal communication, December 8, 2010). Respondents also reported use of the first-generation anticoagulant poison diphacinone, but this chemical is also highly toxic to birds and mammals (Rattner et al. 2011).

Entire housing developments in our study area may contribute to secondary poisoning through systematic use of SGARs. One homeowner noted on their returned survey that her HOA had applied numerous bait stations containing difethialone around homes for many years, but has since changed to a more environmentally friendly method.

We speculate that homeowners with pets may be more wary of using chemical rodenticides; one homeowner stated that "[We] used the poisons before but not anymore because of the cat and also the hawks." This was consistent with Morzillo and Mertig's (2011a) suggestion that concern about rodenticides affecting wildlife was the most significant predictor of the potential for residents to change their pest control behavior.

Stricter U.S. Environmental Protection Agency regulations on pesticides took effect in June 2011 (U.S Environmental Protection Agency 2008). These regulations significantly reduce the availability of SGARs to homeowners by prohibiting their sales in grocery stores, drug stores, and hardware stores. They also specify that these products must be sold in a preloaded bait station or in bulk quantities. Such changes are intended to decrease the potential for exposure of non-target wildlife (U.S. Environmental Protection Agency 2008).

The EPA's mitigation measures contain an implicit assumption that homeowners are more likely than a pest control operator to misuse products, which is consistent with our data (even with our small sample size). If residential users do not follow directions carefully when products are available, reducing availability of SGARs may be an effective action to reduce improper use and subsequent effects on wildlife. It may be beneficial to re-survey homeowners after the effective date of new restrictions to determine if rodent control practices have changed and whether these restrictions are an effective way to reduce homeowner use of SGARs. Licensed applicators may account for a great deal of use of these chemicals, and the use of their services may increase with decreased availability of products to homeowners. Currently, 58% of residents near our study area report self-applying rodent control products (Morzillo and Mertig 2011b), so the EPA rule change may have a substantial effect.

The geography of our study sites limited our ability to distribute fliers easily, and may have contributed to low response rate. Some locations were gated or depositing fliers was not allowed. The homeowner or upkeep staff may not have seen the flier or interpreted it as junk

mail. Therefore, our challenges revealed a difficulty with trying to recruit participants living in affluent areas by media other than mail or telephone.

Some potential biases were unavoidable. First, the title and purpose of the survey may have caused participants to make assumptions about what responses were expected by surveyors. Second, those who are not using rodent control may have felt it unnecessary to participate. Conversely, the UCLA Institute of the Environment as the research group may have led participants choose "environmentally friendly" answers, or to not respond in general. The probability of response may also have been affected by unwillingness to report behavior that might be construed as being irresponsible or illegal and those who have a low level of environmental awareness or interest may not respond either, although eligibility to win a gift certificate was provided as incentive for participation to offset this tendency. Nevertheless, the results do show that off-label use of SGARs does occur, which justifies further investigation.

Future studies should attempt to obtain a greater response rate from both homeowners and PCOs. Regardless, this research yielded: (1) the finding that off-label use was common among respondents, while our very small sample of PCOs reported following guidelines, and (2) information about logistics of surveying by an online questionnaire with participants solicited by fliers delivered to their homes. Although Morzillo and Mertig (2011a, b) had previously investigated what type of chemical products were used and where products were applied, they did not report on whether compounds were first- or second-generation ARs or how exactly residents applied the chemicals. Further research using mailed surveys and multiple follow-up techniques could be used to confirm and generalize the results of our findings and should be expanded to further explore the influence of attitudes about wildlife and potential non-target poisoning (e.g., pets) on SGAR use. Such an approach could also track the effects of the EPA's rule change. It would also be useful to add questions about where residents buy their rodent-control products and inquire about the factors that influence the choice of product. Our results have provided preliminary results that could aid in developing such expanded survey instruments.

To mitigate poisonings now, we recommend outreach programs discussing the potential effects chemical products on wildlife. Near our study area, Morzillo and Schwartz (2011) found relationships between rodent control and resident proximity to natural areas. Thus, for example, property owners next to natural areas and who control rodents also might be gently reminded to review product application directions. Awareness or outreach may solve the problem. Yet, at least two respondents who claimed to know about the adverse effects of SGARs on wildlife also reported using them, so regulation will still be key to any approaches to reduce exposure of non-target species to SGARs.

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APPENDICES

Appendix A: Survey Questionnaire

1. Information sheet for consent to participate in a research study. By reading and accepting this questionnaire, I am agreeing to participate in this study.
   - _ Yes, I agree to participate in this study.
   - _ No, I do not agree to participate in this study.

2. Do you currently live in [survey area]?
   - _ Yes
   - _ No

3. Do you live in a single-family residence?
   - _ Yes
   - _ No

4. Do you live south of Ventura Boulevard?
   - _ Yes
   - _ No

5. Has any form of rodent control been used on your property in the past year?
   - _ Yes
   - _ No

6. What animals are/were you trying to control for? (check all that apply)
   - _ Mice
   - _ Rats
   - _ Gophers
   - _ Moles
   - _ Squirrels
   - _ Opossums
   - _ Raccoons
   - _ Skunks
   - _ Other _____

7. What caused your household to begin controlling these animals on your property? (check all that apply)
   - _ Observed animals indoors
   - _ Observed animals outdoors
   - _ Damage observed to own structures
   - _ Damage observed to neighbor's structures
   - _ Damage observed to own landscaping (including garden, lawn, etc.)
   - _ Damage observed to neighbor's landscaping (including garden, lawn, and etc.)
   - _ Preventative use
   - _ Part of routine treatment by hired company
   - _ Other _____

8. Who applied the rodent control? (check all that apply)
   - _ Member of household
   - _ Pest control company

- _ Gardener/landscape company
- _ Not sure
- _ Other _____

9. If you answered with Pest Control company, please specify which company:
- _ Don't remember
- _ Please specify: _____

10. If you answered with Pest Control company above, did they provide you with information about the products they applied?
- _ Yes
- _ No
- _ Not sure
- _ Not applicable

11. Which, if any, of the following non-chemical rodent control methods have been used on your property in the past year: (check all that apply)
- _ Snap traps
- _ Glue boards
- _ Live traps
- _ Shooting
- _ Electricity (i.e. rat zapper)
- _ Ultrasound deterrents
- _ Preventative methods (e.g. securing access points, cutting vegetation)
- _ Don't know
- _ None

12. [Brand images] Which, if any, of the following brands of chemical rodent control methods have been used on your property in the past year: (check all that apply)
- _ d-con
- _ Tomcat Liquid
- _ Tomcat Bait Stations
- _ Tomcat Quickstrike
- _ Tomcat Pellets, Blocks, and Trays
- _ Moletox
- _ Wilco Baits
- _ Victor Fast-Kill
- _ Victor Multi-Kill
- _ Ratol
- _ FirstStrike
- _ Rodetrol
- _ Other fumigants (e.g. gas canisters)
- _ Other nerve agent (e.g. Bromethalin)
- _ Zinc phospide
- _ Don't know
- _ None
- _ Other

13. If chemical rodent control is applied on your property, how often is it applied?
- _ Approximately every month or more often

_ Approximately every other month (6 times per year)
_ Approximately every four months (3 times per year)
_ Approximately twice a year
_ Approximately once a year or less often
_ Other _____

14. If chemical rodent control is applies on your property, in what locations INSIDE of structures is it used? (check all that apply)
_ Basement
_ Crawlspace
_ Attic
_ Another location within home
_ Garage
_ Outbuilding
_ Not applied
_ Other _____

15. If chemical rodent control is applied on your property, in what locations OUTSIDE structures is it used? (check all that apply)
_ Along walls of any building (within 1 foot)
_ Between 1 and 10 feet from any building
_ Between 10 and 30 feet from any building
_ Between 30 and 100 feet from any building
_ Between 100 and 300 feet from any building
_ More than 300 feet from any building
_ Not applied outside

16. Has anyone in your household found dead animals at the following locations INSIDE structures after chemical rodent control methods have been applied? (check all that apply)
_ Basement
_ Crawlspace
_ Attic
_ Another location within home
_ Garage
_ Outbuilding
_ Not applied
_ Other _____

17. Has anyone in your household found dead animals at the following locations OUTSIDE structures after chemical rodent control methods have been applied? (check all that apply)
_ Along walls of any building (within 1 foot)
_ Between 1 and 10 feet from any building
_ Between 10 and 30 feet from any building
_ Between 30 and 100 feet from any building
_ Between 100 and 300 feet from any building
_ More than 300 feet from any building
_ Not applied outside

18. Are you aware that chemicals used for residential rodent control may be affecting wildlife in your area?

- _ Yes
- _ No

19. Does your household have a pest with access to the outside?
    - _ Yes
    - _ No

20. Does anyone under 18 years old live in your household?
    - _ Yes
    - _ No

21. How large is your property?
    - _ Less than 5,000 square feet (0.1. acre)
    - _ 5,001–7,000 square feet (0.11–0.16 acre)
    - _ 7,001–10,000 square feet (0.17–0.23 acre)
    - _ 10,001–21,779 square feet (0.24–0.49 acre)
    - _ 0.5–1 acre
    - _ More than 1 acre

22. What is your annual household income?
    - _ Less than $50,000
    - _ $50,000 to $75,000
    - _ $75,001 to $100,000
    - _ $100,001 to $150,000
    - _ $150,001 to $200,000
    - _ $200,001 to $300,000
    - _ More than $300,000

23. What is the highest level of education you have completed?
    - _ Less than high school
    - _ High school or FED
    - _ Vocation or trade school
    - _ Some college
    - _ Associate's (2 year) degree
    - _ Bachelor's (4 years) degree
    - _ Graduate or professional degree

24. Please specify your year of birth.

25. What is your ethnic background?
    - _ White/Caucasian
    - _ Black/African American
    - _ Asian/Pacific Islander
    - _ Hispanic/Latino
    - _ Other _____

Thank you for your participation!

If you wish to be entered into a drawing for a $50 Best Buy Gift Card, please email your contact information to [student email]. Your email will not be associated with your responses to the survey and we won't share your email with anyone or send you messages.

Appendix B: Door-to-door Recruitment Flier

UCLA Institute of the Environment Senior Environmental Science Practicum

Methods of Rodent Control in Residential Areas Surrounding the Santa Monica Mountains

The purpose of the survey is to study the reasons for and the use of rodent control methods around the Santa Monica Mountains. The survey is expected to last only 5 – 10 minutes, and your participation is completely voluntary. You may exit at anytime without any consequences, and all data collected in this survey will be kept confidential.

Upon completion of the survey, you will have the option to email us to enter yourself in a drawing to win a $50 Best Buy gift card.

The link for the survey is as follows: **[*website*].**
You will be directed to a UCLA Institute of the Environment Website. Please click on Rodenticide Usage Study to participate in the survey. The deadline to participate in the survey is [*date*]

If you have any questions, feel free to contact [*name*] at [*email*], or Dr. Travis Longcore, our faculty advisor, at longcore@ucla.edu. Thank you for your time.

Appendix C: Pest Control Company Interview Questionnaire

1. What areas does your company currently service?

2. How does your company control for rodents?
    2a. If you use chemical rodent control, which chemicals does your company use?
    2b. If you use physical rodent control, which methods does your company use?

3. Does your company control for _____?
    _ Mice
    _ Rats
    _ Gophers
    _ Moles
    _ Squirrels
    _ Opossums
    _ Raccoons
    _ Skunks
    _ Other _____

4. Do your customers tell your company why they need rodent control?
    _ If so, what are the main reasons you hear?

5. What information does your company provide to customers regarding rodent control?

6. How often do you apply/reapply rodenticides at an average household?

7. Does your company apply rodent control inside structures?
    _ If so, where? (Garage, basement, crawl space, attic, etc.)

8. Does your company apply rodent control outside structures?
    _ If so, at what distances from buildings?

About

ONE VOICE SINCE 1898

For more than 125 years, the California Grocers Association has served as the voice of the state's grocery community. A nonprofit, statewide trade association, CGA's membership is comprised of over 300 retailers operating more than 6,000 brick-and-mortar stores, and approximately 150 grocery supply companies.

The Association is recognized as the leading resource in the areas of government and industry relations for California's food industry and its customers while also providing continuing education opportunities for industry employees and their children.

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Northern spotted owl | Dave Roelofs, BLM

PESTICIDE EXPOSURES & MORTALITIES IN NON-TARGET WILDLIFE

CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

2022 Annual Report
Wildlife Hea lth Laboratory
31 July 2023

2022 SUMMARY OF PESTICIDE EXPOSURES & MORTALITIES IN NON-TARGET WILDLIFE

By Jaime Rudd, Krysta Rogers, & Nicholas Shirky

With contributions from Deana Clifford and Brandon Munk

PREPARED BY THE WILDLIFE HEALTH LABORAOTRY OF THE CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

State of California
Natural Resources Agency
INTRODUCTION

It is the mission of the California Department of Fish and Wildlife (CDFW) to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. As such, a memorandum of understanding was developed between the California Department of Pesticide Regulation (CDPR), the County Agriculture Commissioners (CAC), and the CDFW. The purpose of the memorandum is to ensure that pesticides registered in the state of California are used in a manner that protects non-target fish and wildlife resources, while recognizing the need for responsible pest control.

In partial fulfillment of the MOU, this 2022 annual report summarizes documented pesticide exposure and toxicosis in California's fish and wildlife for the respective authorities of CDPR, CAC, and CDFW. These data represent a minimum number of reports for tested animals that died within the reported calendar year and are subject to change as new information becomes available.

DATA COLLECTION & ANALYSIS

The Wildlife Health Laboratory (WHL, formerly the Wildlife Investigations Laboratory) was established in 1941 and is mandated by Fish and Game Code Section 1008 to investigate all diseases and problems relating to wildlife. The WHL has accomplished this goal through collaboration with the public and various organizations to record, collect, and submit wildlife mortalities of interest to the WHL for examination and further diagnostics as needed. The WHL continues communication with interested parties as new information is discovered to aid further cooperation in the goal of maintaining healthy wildlife populations throughout the state.

Programmatically the WHL is divided into three units which address health issues: 1) avian, 2) large game, 3) small and non-game species. The avian unit oversees nearly 600 avian species including non-game (e.g., songbirds, raptors, shorebirds, waders, and seabirds) and game species (e.g., doves, pigeons, quail, turkey, and waterfowl). The large game unit primarily oversees black bear, bighorn sheep, deer, elk, pronghorn, and wild pig with shared responsibility of small game such as tree squirrels, rabbits, and hares. In addition to sharing health surveillance responsibilities with the large game unit, the non-game unit also oversees native non-game mammals, fur bearers, reptiles, and amphibians. This includes a consortium of species such as California tiger salamander, Western Pond turtles, pika, riparian brush rabbits, skunks, raccoons, foxes, bobcats, mountain lions, and gray wolves.

Wildlife Submissions

Wildlife remains are submitted to the WHL in various ways, primarily by the public – either direct submissions of deceased wildlife to the WHL, submission of living or deceased wildlife to wildlife rehabilitation centers ("rehab"), notification of mortalities to CDFW staff and law enforcement, or other government agency reports (e.g., animal control, sheriff, state and federal Department of Agriculture, U.S. Fish and Wildlife Service, the Park Service, etc.). The WHL also collaborates with universities, non-governmental organizations (NGO), and other agencies on statewide population monitoring projects and provides diagnostic support by conducting postmortem examinations. The WHL contracts with the California Animal Health and Food Safety (CAHFS) Laboratory for further disease and toxicology testing.

Postmortem Examination

Postmortem examinations (necropsies) are performed on wildlife remains at the WHL or the CAHFS Laboratory. If remains cannot be examined within 48-hours of collection, they are stored in a -20°C freezer until an examination can be performed. Prior to necropsy, frozen carcasses are thawed for a few days at 4°C or room temperature until they are ready for necropsy. Sex, age class, body condition and, when

possible, the cause of death is determined. In addition to necropsy, mortality investigations often include microscopic evaluation of tissues (histology) and ancillary disease and toxicology testing. Tissue samples are collected and placed in 10% formalin for histological evaluation and a complimentary set of tissues are archived in -20C° freezers until submitted to the CAHFS Laboratory for analysis.

Carcasses in advanced stages of decomposition and autolysis are necropsied but formalin tissues may not be collected or submitted since autolysis can obscure or destroy microscopic lesions. In these cases, necropsies are performed, and tissue samples are collected for toxicology testing to rule out pesticide exposure but not necessarily toxicosis.

Anticoagulant Rodenticides. Anticoagulant rodenticides are grouped into two categories: "first generation anticoagulant rodenticides" which include warfarin (war), coumachlor (cou), diphacinone (diph), and chlorophacinone (chl) and the more toxic "second generation anticoagulant rodenticides" which include brodifacoum (brd), bromadiolone (brm), difenacoum (dfn), and difethialone (dif).

Liver samples are submitted to the CAHFS Laboratory for testing.

Non-Anticoagulant Rodenticides & Other Pesticides. A number of acutely toxic compounds such as bromethalin, strychnine, zinc phosphide, cholecalciferol, organophosphates, and carbamates are also used to manage rodent and insect pests. Like anticoagulant rodenticides, these compounds, or their metabolites, have been documented in non-target wildlife as a form of mortality or exposure.

Appropriate tissue samples (e.g., gastrointestinal contents, adipose, brain, spinal cord, kidney, liver) for requested tests are also submitted to the CAHFS Laboratory for testing.

Exposure & Toxicosis

Pesticides, including anticoagulant rodenticides, are not always acutely fatal and there is a high degree of variability among species and individuals in their vulnerability. In the absence of a universal threshold residue value that could indicate anticoagulant rodenticide "toxicosis," we must also rely on antemortem and/or postmortem evidence of coagulopathy unrelated to another identifiable cause of hemorrhage (e.g., trauma, disease, infection).

Individuals are considered to have anticoagulant rodenticide "exposure" if their livers had detectable levels of one or more anticoagulant rodenticide residues (regardless of concentration, reported in parts per billion or ppb) and lack antemortem and/or postmortem evidence of coagulopathy.

For non-anticoagulant rodenticides, diagnosing toxicosis requires the detection of the compound in the appropriate tissue sample or gastrointestinal contents, and antemortem and/or postmortem evidence in the absence of another identifiable cause (e.g., disease, infection, trauma).

In some cases, rodenticide residues are detected in the tissue sample, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition which precludes a definitive diagnosis. Therefore, these diagnoses are reported as "suspected" or "undetermined" toxicosis.

It is important to note that exposure in the absence of toxicosis should not be ignored[1]. The uncertainties about the magnitude and drivers of chronic exposure and/or sub-lethal levels of rodenticide exposure demonstrate the need for continued monitoring. Exposure to anticoagulant rodenticides may predispose wildlife to excessive hemorrhage following an otherwise non-lethal traumatic injury or increase sensitivity to additional exposure(s)[1].

AVIAN SUMMARY

According to CDFW records at the time of this report, the remains of 1,211 birds were submitted to the WHL for necropsy, and/or disease or toxicology testing in calendar year 2022. Note, the number of birds submitted to WHL in 2022 was roughly twice the average number of birds submitted in previous years. The primary reason for increased submissions during 2022 was the unprecedented outbreak of Eurasian highly pathogenic avian influenza H5N1 that affected a diversity of wild birds and poultry in California, elsewhere in the United States, and globally. The ability to conduct surveillance testing for other diseases and exposure to toxins was impacted by the demand for disease testing for highly pathogenic avian influenza H5N1. Further, highly pathogenic avian influenza viruses are designated as a United States Department of Food and Agriculture select agent and a reportable foreign animal disease. All tissues are required to be immediately disposed of following a confirmed detection to reduce the risk of disease spread, and thus no further testing could be performed.

Waterfowl and waterbirds (n = 563) accounted for the largest percentage of birds submitted, followed by raptors (n = 438). Birds were submitted for various reasons by wildlife rehabilitators, members of the public, non-profit organizations, universities, CDFW staff and law enforcement, and other agencies (Table 1). Wildlife rehabilitators made up the majority of submissions, followed by agencies and specifically, CDFW. However, it should be noted that the majority of these reports originated with a member of the public.

Table 1. Total number of wild bird remains submitted to the Wildlife Health Laboratory for necropsy in 2022 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Birds Submitted
CDFW	198
NGO/Non-Profit	41
Other Government Agency / Military	71
Private Consultant / Energy	37
Public	38
Rehab / Zoo / Sanctuary	823
University Affiliate	3
Total	**1,211**

Anticoagulant Rodenticide Exposure & Toxicosis

Of necropsied birds, 34 were tested for anticoagulant rodenticide exposure. Tested birds represent 95% (55/58) of California counties (Table 2). All age classes and sexes were represented in submitted carcasses.

Raptors were the largest group to have anticoagulant rodenticide exposure to one or more analyte(s) and/or toxicosis (Table 3). Of the 88.2% of tested birds with detectable levels of anticoagulant rodenticides (30/34), 56.7% (17/30) were cases of anticoagulant rodenticide toxicosis.

More than half of the exposed raptors had two or more second generation anticoagulant rodenticides detected in the liver (Figure 1). Brodifacoum, bromadiolone, difethialone, and diphacinone were the

most common analytes detected in liver samples (Figure 2). None of the birds sampled had detectable levels of exposure to warfarin, difenacoum, or coumachlor.

Other Pesticides

Other pesticide-related investigations involved five separate incidents of mortality including 1) a mourning dove in Sacramento County, 2) rock pigeons in Fresno County, 3) rock pigeons in San Mateo County, 4) a great horned owl in San Luis Obispo County, and 5) a red-tailed hawk in Sonoma County. Avitrol was detected in a rock pigeon submitted from Fresno and San Mateo counties where multiple pigeons were reported with seizures before death. Avitrol was also detected in a single mourning dove reported with seizures before death and submitted from Sacramento County. Strychnine was detected in a great horned owl from San Luis Obispo County and a red-tailed hawk from Sonoma County. The great horned owl had the remains of a songbird in its digestive tract and the red-tailed hawk had the remains of a mourning dove in its digestive tract. The ingested birds were the presumed source of secondary exposure for these raptors as their remains were admixed with strychnine bait in the raptors digestive tract.

American kestrel | Naser Mojtahed, USFWS

Table 2. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 34 tested wild birds submitted to the Wildlife Health Laboratory in 2022 by county. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

County	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
Contra Costa	1	1	100.0	1
Kern	1	1	100.0	1
Los Angeles	5	5	100.0	4
Marin	3	2	66.7	2
Mendocino	2	1	50.0	0
Napa	1	1	100.0	0
Sacramento	4	4	100.0	0
San Bernardino	2	2	100.0	1
San Diego	2	2	100.0	1
San Joaquin	1	1	100.0	0
San Luis Obispo	2	2	100.0	0
San Mateo	1	1	100.0	1
Santa Clara	3	3	100.0	2
Santa Cruz	1	1	100.0	1
Sonoma	2	1	50.0	1
Ventura	3	2	66.7	2
Total	**34**	**30**	**88.2**	**17**

Table 3. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 34 wild birds submitted to the Wildlife Health Laboratory in 2022 by species (common name). After a postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Bird Species	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
American kestrel	1	0	0.0	0
Barn owl	5	5	100.0	2
Golden eagle	2	2	100.0	0
Great horned owl	16	14	87.5	12
Red-shouldered hawk	4	4	100.0	2
Red-tailed hawk	2	2	100.0	1
Swainson's hawk	1	1	100.0	0
Turkey vulture	3	2	66.7	0
Total	**34**	**30**	**88.2**	**17**

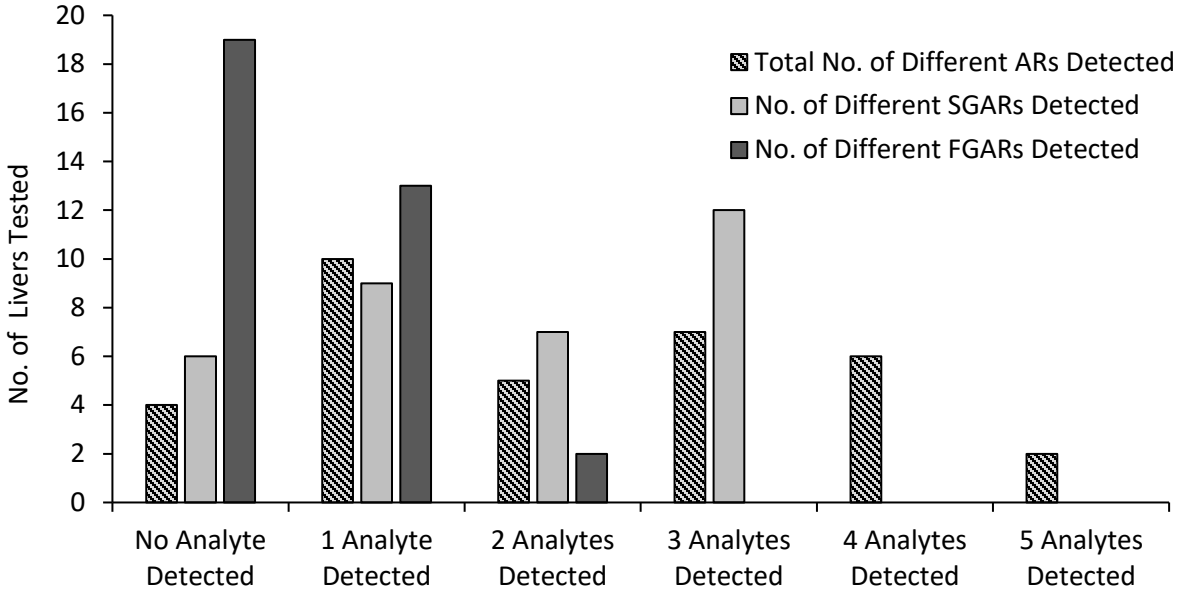

Figure 1. Number of anticoagulant rodenticide residues detected in the livers of 30 wild birds submitted to the Wildlife Health Laboratory for postmortem examination in 2022. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

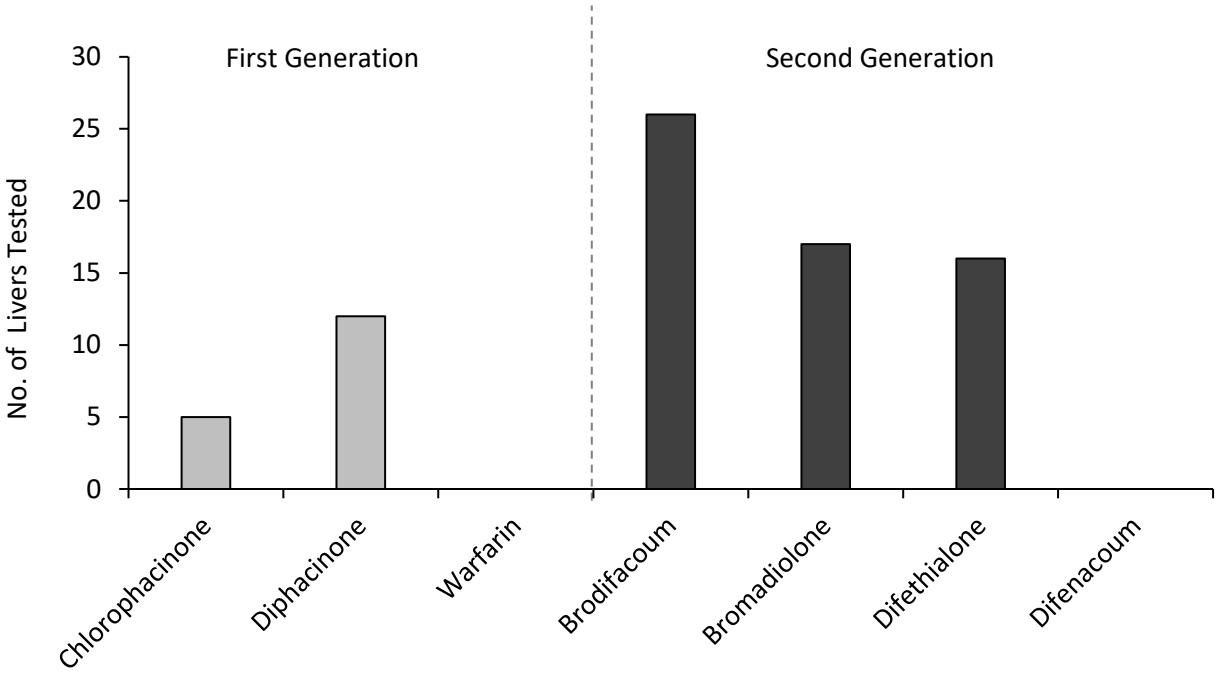

Figure 2. Anticoagulant rodenticide residues detected in the livers of 30 of the 34 tested wild birds submitted to the Wildlife Health Laboratory in 2022. Anticoagulant rodenticides were not detected in 4 of the tested bird livers. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

LARGE GAME SUMMARY

The remains and/or tissues of 68 large game mammals were submitted to the WHL for necropsy and/or toxicology testing in the year 2022.

Approximately 81% (55/68) of the large game carcasses were submitted by the CDFW and other agencies (Table 4). However, it should be noted that public reports represent the original source for most CDFW submissions.

Table 4. Total number of wild large game mammal tissues or remains submitted to the Wildlife Health Laboratory in 2022 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Large Game Mammals Submitted
CDFW	55
Other Government Agency / Military	1
Private Consultant / Energy	1
Public	3
Rehab/Zoo/Sanctuary	8
Total	68

Anticoagulant rodenticides

Of necropsied large game mammals, 15 were tested for anticoagulant rodenticide exposure. Large game mammals were submitted from 11 of the 58 counties in California (Table 5). All age classes and sexes were represented in submitted carcasses.

Black bears accounted for the majority of large game mammals submitted with anticoagulant rodenticide exposure (Table 6). In total, 12 of the 15 (80%) large game mammals tested had exposure to one or more anticoagulant rodenticide and almost half of the tested animals (46.7%, 7/15) had exposure to two or more anticoagulant rodenticides regardless of first- or second generation (Figure 3). One sub-adult female from El Dorado County had exposure to five different anticoagulant rodenticides.

Diphacinone and brodifacoum were the most common analytes detected in tested liver samples (Figure 4). Coumachlor was not detected in any of the submitted liver samples.

None of the 12 exposures resulted in cases of anticoagulant rodenticide toxicosis.

Other Pesticide Exposure

Adipose from 14 black bears and one wild pig, and liver from one black bear from nine California counties were tested for exposure to the neurotoxic rodenticide, bromethalin (Table 7 and 8). Three of the tested black bears and the wild pig had detectable levels of bromethalin in the submitted samples. Of the four cases where bromethalin was detected, toxicosis was determined to be the cause of death in a young black bear from Kern County with a history of ataxia, circling, and incoordination. The bear was found deceased and submitted for postmortem examination and toxicology testing at the California Animal Health and Food Safety Lab in Tulare. Segmental mild vacuolation at the grey/white mater interface of the brain and chronic demyelination with Bungner's bands of motoric nerves fibers were

observed of the cauda equina nerve roots in the lumbar and sacral region with no other associated pathogens or injuries.

Two bears from El Dorado County were tested for exposure to organophosphates; no detectable levels were found.

A general toxicology panel (GMCS/LCMS) was performed on a black-tailed deer from Nevada County. Caffein was detected in the submitted liver sample.

Acetylcholinesterase activity was measured as within normal limits for two bears from Los Angeles and El Dorado County, and black-tailed deer from Tehama County.

Samples of blue-colored adipose (fat), muscle, and brain from an adult female black bear taken under a hunting permit in Sierra County were submitted for rodenticide testing. The sample was screened for the presence of anticoagulant rodenticide residues, and diphacinone was detected in all three of the tested samples. Exposure to other anticoagulant rodenticides or other pesticides cannot be ruled out, however, because liver is the preferred sample for anticoagulant rodenticide testing.

Table 5. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 15 tested wild large game mammals submitted to the Wildlife Health Laboratory in 2022 by county. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

County	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
El Dorado	3	3	100.0	0
Humboldt	1	0	0	0
Kern	1	1	100.0	0
Los Angeles	1	1	100.0	0
Madera	1	1	100.0	0
Nevada	1	1	100.0	0
Placer	1	1	100.0	0
San Bernardino	2	2	100.0	0
Siskiyou	2	1	50.0	0
Tehama	1	0	0	0
Ventura	1	1	100.0	0
Total	**15**	**12**	**80.0**	**0**

Table 6. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 15 wild large game mammals submitted to the Wildlife Health Laboratory in 2022 by species. After a postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Large Game Species	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
Black bear	13	11	84.6	0
Black tailed deer/ Mule deer	1	0	0	0
Wild pig	1	1	1	0
Total	**15**	**12**	**80.0**	**0**

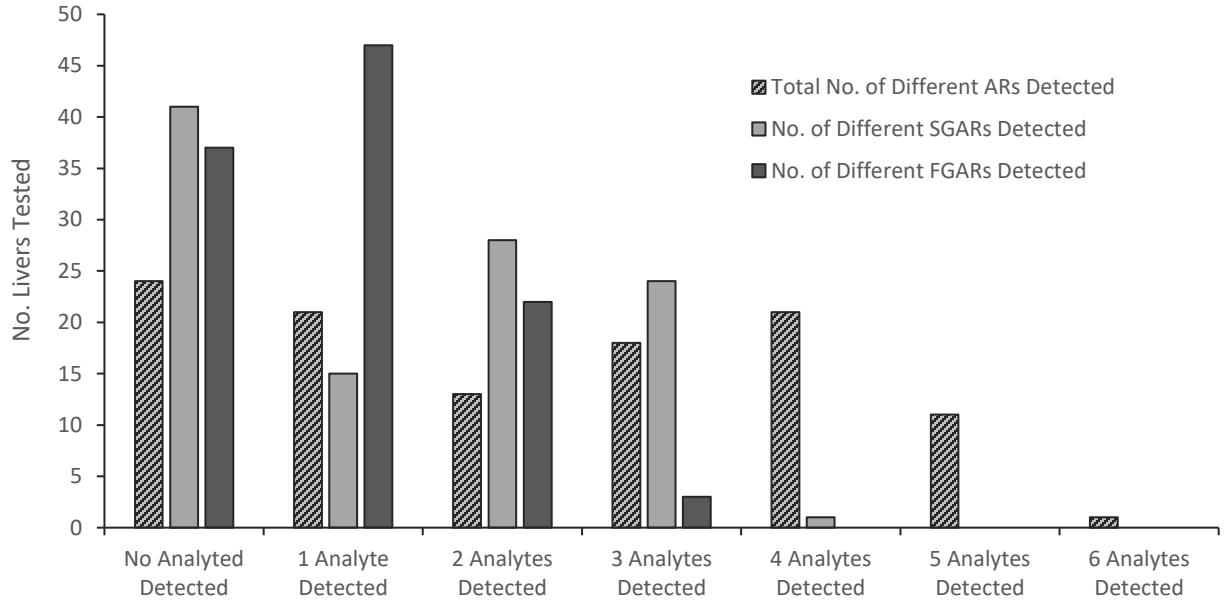

Figure 3. Number of anticoagulant rodenticide residues detected in the livers of 15 wild large game mammals submitted to the Wildlife Health Laboratory for postmortem examination in 2022. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

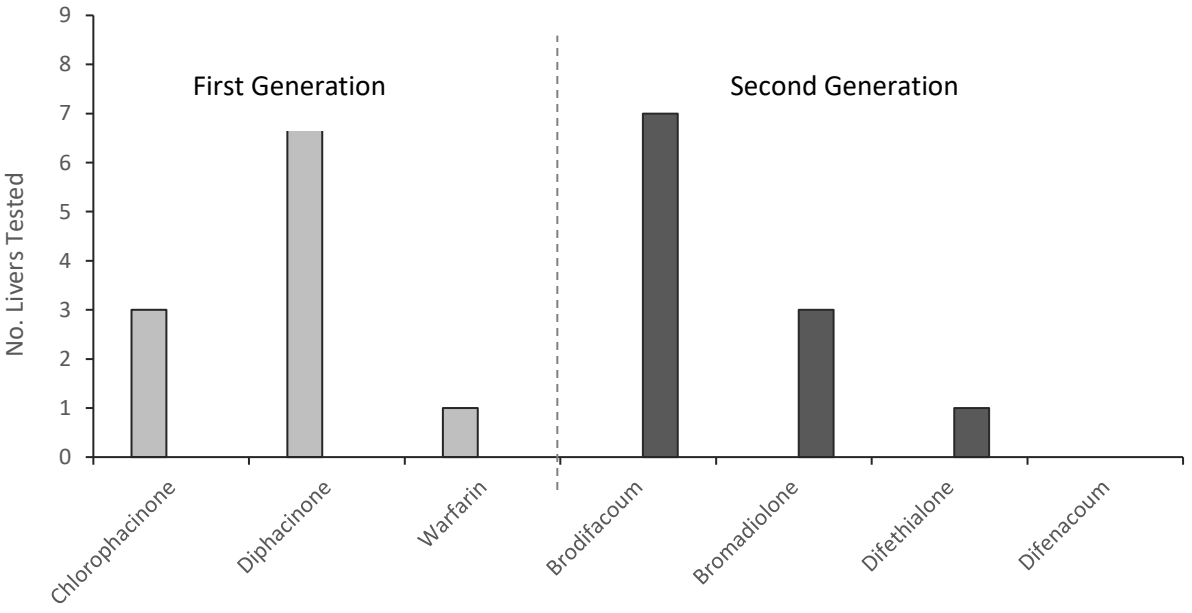

Figure 4. Anticoagulant rodenticide residues detected in the livers of 12 of the 15 tested wild large game mammals submitted to the Wildlife Health Laboratory in 2022. Anticoagulant rodenticides were not detected in 3 of the tested large game mammal livers. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Table 7. Bromethalin exposure in wild large game mammals submitted to the Wildlife Health Laboratory in 2022 by county. Adipose or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

County	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
El Dorado	5	1	20.0	0
Kern	1	1	100.0	1
Los Angeles	1	0	0	0
Madera	1	1	100.0	0
Nevada	1	0	0	0
Placer	1	0	0	0
San Bernardino	2	1	50.0	0
Siskiyou	2	0	0	0
Ventura	1	0	0	0
Total	15	4	26.7	0

Table 8. Bromethalin exposure in wild large game mammals wildlife submitted to the Wildlife Health Laboratory in 2022 by species. Adipose or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Species	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis
Black bear	14	3	21.4	1
Wild pig	1	1	100.0	0
Total	**15**	**4**	**26.7**	**0**

American black bear | Eileen Hornbaker, USFWS

SMALL GAME & NON-GAME SUMMARY

The remains of 264 herptiles and mammals were submitted to the WHL for necropsy in 2022. This included samples and remains of animals primarily for specialized disease surveillance such as rabbit hemorrhagic disease virus (lagomorphs), snake fungal disease (snakes), and white-nose syndrome (bats).

Small game and non-game animals were submitted for various reasons by wildlife rehabilitators, members of the public, non-profit organizations, universities, CDFW staff and law enforcement, and other agencies. Wildlife rehabilitators made up 35% (92/264) of submissions, followed by CDFW (33%; Table 9). Toxicology testing was not performed on the herptiles. Therefore, the remainder of this section will address completed test results for mammals.

Table 9. Total number of wild small- and non-game mammal remains submitted to the Wildlife Health Laboratory in 2022 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Small- and Non-Game Animals Submitted
Animal Control	9
CDFW	87
NGO/Non-Profit	3
Other	2
Other Government Agency	14
Private Biological Consultant	2
Public	21
Rehab/Zoo/Sanctuary	92
University Affiliate	34
Total	**264**

Anticoagulant Rodenticide Exposure & Toxicosis

Of necropsied mammals, 150 were tested for pesticide exposure but results are only available for 109 tested mammals at the time of this report. Sampled remains with final reports represent 38 of the 58 counties in California (Table 10). The remains for a juvenile mountain lion did not have a specified location. All age classes and sexes were represented.

Bobcats accounted for the largest percentage of mammal samples submitted to the WHL (Table 11). In total, 86 of the 109 (78.9%) mammals tested had exposure to one or more anticoagulant rodenticide and almost half of the tested animals had exposure to three or more anticoagulant rodenticides regardless of first- or second generation (Figure 5). One adult female bobcat from Orange County had exposure to six different anticoagulant rodenticides.

One of the 86 exposures (1.2%) resulted in a case of anticoagulant rodenticide toxicosis (Table 11). Anticoagulant rodenticide toxicosis was suspected in 3.5% (3/86) of tested animals with livers that had detectable residue exposure, however toxicosis could not be ruled in or out in due to advanced stages of decomposition, making gross and histological interpretation of the tissues difficult.

Brodifacoum, bromadiolone, and diphacinone were the most common analytes detected in liver samples (Figure 6). None of the tested samples had detectable levels of exposure to coumachlor.

Other Pesticide Exposure

One-hundred three wild non-game and small game mammals were tested for additional pesticides, including bromethalin, organophosphates and carbamates, neonicotinoids, pyrethroids, fipronil and fipronil sulfone.

Adipose or brain from 95 animals across 34 counties was tested for exposure to the neurotoxic rodenticide, bromethalin (Table 12). Twenty-two of the tested animals had exposure to bromethalin and 22.7% of those exposures resulted in mortality (2/22) or suspected mortality (3/22) (Tale 13). Advanced decomposition likely precluded the identification of any lesion(s) that may be associated with bromethalin toxicity in the long-tailed weasel with exposure. Further, it had a clinical history of depressed behavior with possible neurologic signs prior to death but these signs were not described in detail by the submitter. Thus, it is undetermined if exposure may have resulted in clinical signs and toxicosis.

A general toxicology panel (GMCS/LCMS) was performed on two raccoons from Sonoma and Tehama Counties. No toxic compounds were detected.

Vitamin D3 levels were tested in a mature adult female bobcat after tubular mineralization was observed in the vessels of her lungs and kidneys to rule out Vit-D3 toxicosis. Vitamin D3 levels were within normal limits and the mineralization observed is suspected to have been non-clinically significant.

Twelve North American river otters were tested for neonicontinoids, pythreins, fipronil and fipronil sulfone, and organophosphates, however final results are only available for five river otters at the time of this report. None of the toxic compounds were detected.

American badger | Tom Koerner, USFWS

Table 10. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in the livers of 109 small game and non-game remains submitted to the Wildlife Health Laboratory for postmortem examination in 2022 by county. Livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, rodenticide residues were detected in the liver, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition. Therefore, these diagnoses are reported as "undetermined" toxicosis.

County	No. tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis	No. Undetermined Toxicosis
Butte	1	1	100.0	0	0
Calaveras	2	1	50.0	0	0
Contra Costa	6	4	66.7	0	0
El Dorado	1	1	100.0	0	0
Fresno	1	0	0	0	0
Imperial	1	1	100.0	0	0
Inyo	1	1	100.0	0	0
Kern	8	7	87.5	0	0
Kings	1	1	100.0	0	0
Los Angeles	4	3	75.0	0	0
Mendocino	3	3	100.0	0	0
Merced	1	1	100.0	0	0
Modoc	1	0	0	0	0
Mono	7	6	85.7	0	0
Monterey	7	4	57.1	0	1
Napa	2	1	50.0	0	0
Nevada	3	3	100.0	0	0
Orange	6	6	100.0	0	0
Placer	2	2	100.0	0	0
Plumas	2	1	50.0	0	0
Riverside	2	2	100.0	0	0
Sacramento	3	3	100.0	0	1
San Benito	2	1	50.0	0	0
San Bernardino	2	2	100.0	0	0
San Diego	2	2	100.0	0	0
San Francisco	2	2	100.0	0	0
San Joaquin	3	0	0	0	0
San Luis Obispo	1	1	100.0	0	0
San Mateo	8	6	75.0	0	0
Santa Barbara	1	1	100.0	0	0
Santa Clara	3	3	100.0	1	0
Santa Cruz	3	3	100.0	0	0
Shasta	1	1	100.0	0	0
Sierra	2	1	50.0	0	0
Sonoma	8	8	100.0	0	0
Stanislaus	1	0	0	0	0
Tehama	1	1	100.0	0	0
Ventura	3	1	33.3	0	0

County	No. tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis	No. Undetermined Toxicosis
Not specified	1	1	100.0	0	1
Total	**109**	**86**	**78.9**	**1**	**3**

Table 11. Exposure prevalence and toxicosis of anticoagulant rodenticide residues detected in the livers of 109 small game and non-game mammals submitted to the Wildlife Health Laboratory for postmortem examination in 2022 by species. Livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, rodenticide residues were detected in the liver, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition. Therefore, these diagnoses are reported as "undetermined" toxicosis.

Species	No. Tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis	No. Undetermined Toxicosis
Badger	1	1	100.0	0	0
Bobcat	38	33	86.8	0	0
Brush rabbit	5	0	0	0	0
Coyote	6	6	100.0	1	0
Eastern fox squirrel	1	0	0	0	0
Gray fox	13	12	92.3	0	2
Mountain Lion	19	17	89.5	0	1
Raccoon	7	3	42.9	0	0
Red fox	2	1	50.0	0	0
Ringtail	1	0	0	0	0
River otter	5	3	60.0	0	0
San Joaquin kit fox	8	7	87.5	0	0
Striped skunk	3	3	100.0	0	0
Total	**109**	**86**	**78.9**	**1**	**3**

American river otter | Keenan Adams, USFWS

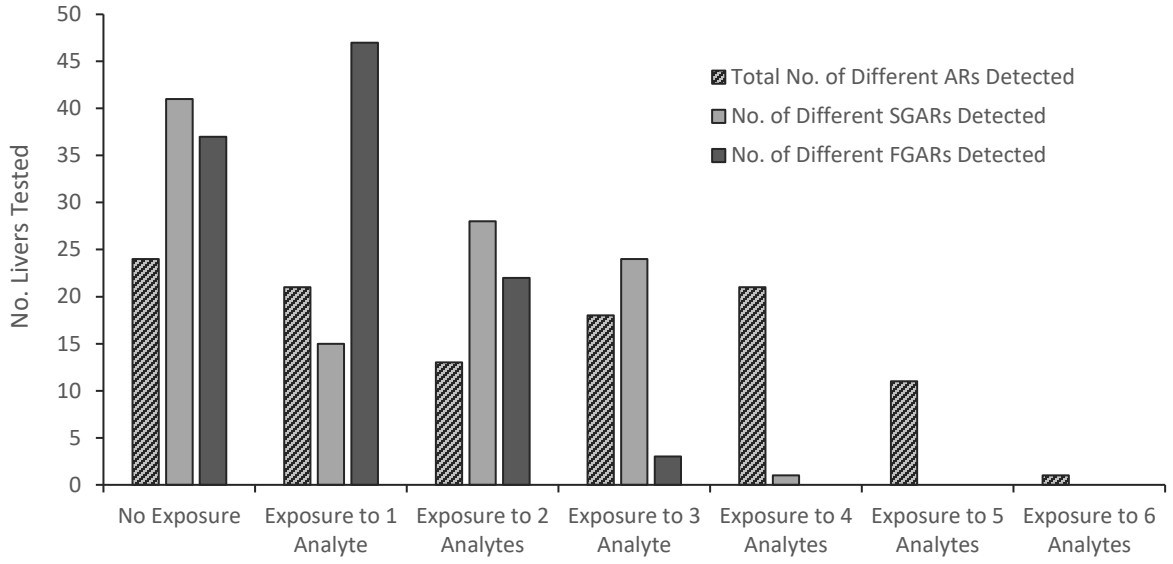

Figure 5. Number of anticoagulant rodenticide residues detected in the livers of 109 small game and non-game mammals submitted to the Wildlife Health Laboratory for postmortem examination in 2022. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

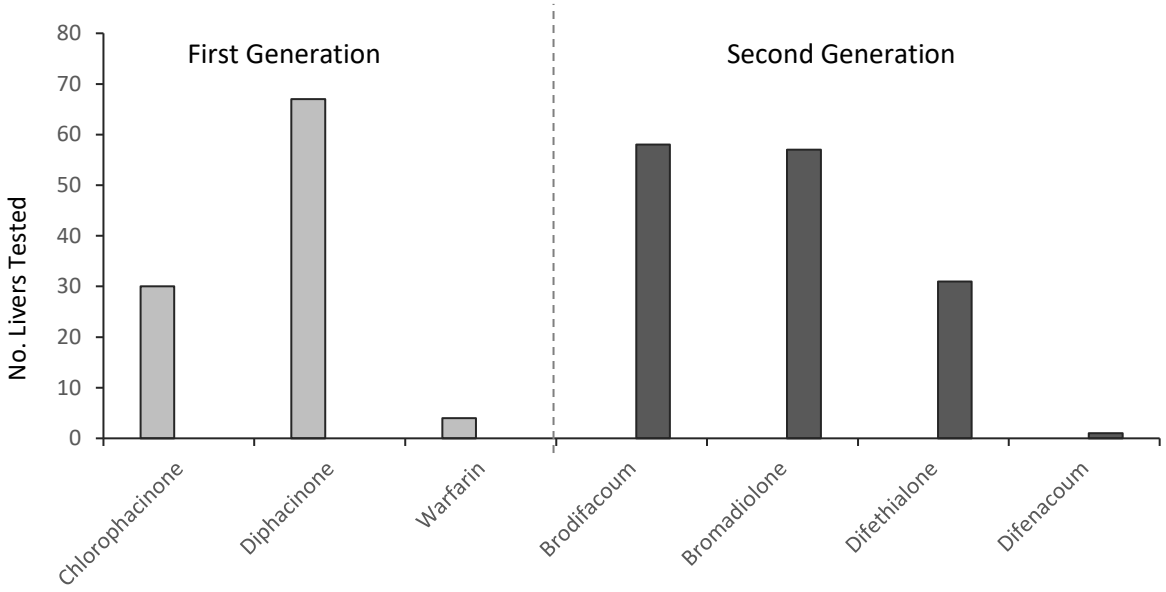

Figure 6. Anticoagulant rodenticide residues detected in the livers of wild small game and non-game mammals submitted to the Wildlife Health Laboratory for postmortem examination in 2022. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Table 10. Bromethalin exposure and toxicosis in wild small game and non-game wildlife submitted to the Wildlife Health Laboratory in 2022 by county. Adipose or brain were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, bromethalin were detected in but antemortem and postmortem evidence could not confirm or exclude toxicosis due to advanced autolysis which may preclude histologically significant lesions or the inability to observe the animal while alive. Therefore, these diagnoses are reported as "undetermined toxicosis."

County	No. tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis	No. Undetermined Toxicosis
Butte	1	0	0	0	0
Calaveras	2	0	0	0	0
Contra Costa	3	1	33.3	0	0
El Dorado	1	0	0	0	0
Fresno	1	0	0	0	0
Imperial	1	0	0	0	0
Kern	7	0	0	0	0
Los Angeles	4	1	25.0	0	0
Marin	1	1	100.0	0	0
Mendocino	3	2	66.7	0	0
Modoc	1	1	100	0	0
Mono	5	0	0	0	0
Monterey	7	2	28.6	0	0
Napa	2	0	0	0	0
Nevada	3	0	0	0	0
Orange	6	3	50.0	0	0
Placer	2	1	50.0	0	0
Plumas	1	0	0	0	0
Riverside	2	0	0	0	0
Sacramento	1	0	0	0	0
San Benito	2	0	0	0	0
San Bernardino	2	0	0	0	0
San Diego	2	0	0	0	0
San Luis Obispo	1	0	0	0	0
San Mateo	7	2	28.6	0	0
Santa Barbara	2	0	0	0	0
Santa Clara	3	0	0	0	0
Santa Cruz	3	0	0	0	0
Shasta	1	0	0	0	0
Sierra	2	0	0	0	0
Sonoma	11	6	54.5	2	3
Tehama	1	0	0	0	0
Tulare	1	0	0	0	0
Ventura	2	1	50.0	0	0
Not specified	1	1	100.0	0	0
Total	**95**	**22**	**23.2**	**2**	**3**

Table 11. Bromethalin exposure and toxicosis in wild small game and non-game wildlife submitted to the Wildlife Health Laboratory in 2022 by species. Adipose or brain were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, bromethalin were detected in but antemortem and postmortem evidence could not confirm or exclude toxicosis due to advanced autolysis which may preclude histologically significant lesions or the inability to observe the animal while alive. Therefore, these diagnoses are reported as "undetermined toxicosis."

Species	No. tested	No. Exposed	Percent Exposed	No. Confirmed Toxicosis	No. Undetermined Toxicosis
Badger	2	0	0	0	0
Beaver	1	0	0	0	0
Bobcat	36	4	11.1	0	0
Coyote	6	1	16.7	0	0
Eastern fox squirrel	1	0	0	0	0
Eastern gray Squirrel	1	0	0	0	0
Gray fox	11	4	36.4	0	2
Mountain lion	17	6	35.3	0	0
Opossum	1	0	0	0	0
Raccoon	8	5	62.5	1	1
Red fox	1	1	100.0	0	0
Ringtail	1	0	0	0	0
San Joaquin kit fox	7	0	0	0	0
Striped skunk	2	1	50.0	1	0
Total	**95**	**22**	**23.2**	**2**	**3**

Raccoon | Bill Buchanan, USFWS

ADDITIONAL SURVEILLANCE

Poisoning of domestic dog

The CDFW was asked to investigate the mortality of a turkey vulture and two dogs on private property. The property owner reported finding her pet dog deceased outdoors near what appeared to be meat left out on a black tray that contained a blue substance and a white plastic container full of yellow liquid. The suspicious meat and liquid were placed along the fence line of the reporting party's property and a neighbor. The property owner buried her pet but found a deceased stray dog and turkey vulture on her property the following day. The property owner reported that the stray dog had foam coming from its mouth, a bloody nose, and vomit next to the dog. By the time CDFW LE officers were contacted, the suspicious meat and yellow liquid had been removed. Brain and stomach contents from the deceased stray dog were collected and submitted to the California Animal Health and Food Safety Lab in Davis. Methomyl, a carbamate insecticide, was detected in the stomach contents. Signs of carbamate toxicosis include hypersalivation, gastrointestinal hypermotility, abdominal cramping, vomiting, diarrhea, dyspnea, cyanosis, miosis, muscle fasciculations (in extreme cases, tetany followed by weakness and paralysis), and convulsions. Death usually results from respiratory failure and hypoxia due to bronchoconstriction leading to tracheobronchial secretion and pulmonary edema[2,3]. Pathological findings of toxicosis include dried saliva around the oral cavity and on other parts of the body that an animal may have touched with their mouth (e.g., forelegs), epistaxis, diffuse uveal congestion and hyphema, subcutaneous and muscular hemorrhage, food with carbamate in the stomach, microhemorrhages in the lower gastrointestinal tract, hemorrhagic pericardial content, diffuse cardiac hemorrhage, diffuse upper respiratory congestion and bilateral pulmonary congestion and edema of the lungs[3]. According to the U.S. Environmental Protection Agency, "There are no residential uses of methomyl. All methomyl products, except the bait formulations, are classified as Restricted Use Pesticides (RUPs). RUPs can only be used by or under the direct supervision of specially trained and certified applicators[4]." In California, a permit is required for the use and application of restricted materials, which includes carbamates such as methomyl[5].

Carbamate insecticides act similarly to organophosphate insecticides and inhibit cholinesterase activity, however cholinesterase activity levels in the brain were elevated. Elevate levels are of unknown clinical significance, however postmortem examination of the dog's remains were consistent with carbamate toxicosis (e.g., hypersalivation, vomiting, pulmonary edema, and hemorrhaging).

No toxic compounds were detected in the turkey vulture by gas chromatography - mass spectrometry (GC/MS) and liquid chromatography - mass spectrometry (LC/MS) organic chemical screens.

Evaluation of Assembly Bill 1788

A temporary moratorium was placed on the public sales and use of second generation anticoagulant rodenticides (SGARs) on January 1, 2021 under AB1788. Given the long half-lives of many SGARs and their ability to bioaccumulate in the livers of living animals, evaluating any immediate changes resulting from this temporary moratorium may be difficult. The CDFW proposed guidelines for monitoring the short-term, immediate effects of AB1788s as well as the continued long-term monitoring and surveillance of anticoagulant rodenticide exposure in non-target wildlife, especially given the special exceptions to this moratorium that still allow for SGAR use.

Short-term evaluation of the efficacy of AB1788 include looking at animals born or hatched after January 1, 2021 and cases of exposure and/or acute toxicosis. Our reasoning is that most wildlife born or hatched after implementation of AB1788 should not have exposure to SGARs (although there is a chance that mammals could have been exposed in utero[6-12]). A study by CDFW looking at anticoagulant rodenticide exposure in mountain lions found that cubs are less likely to have SGAR exposure when compared to adults[12] despite evidence of fetal exposure[6]. Further, we posit that wildlife that have died from acute toxicosis were likely recently exposed at concentrations large enough to cause coagulopathy and death rather than chronic exposure accumulating over time. It is important to note, however, that most wildlife have more than one analyte detected in their livers belonging to both first generation and second generation anticoagulant rodenticides. Additionally, there is no minimum threshold concentration indicative of anticoagulant rodenticide toxicosis and determining whether toxicosis was due to a first generation or second generation is difficult in the presence of multiple analytes and lack of information on the cumulative effects.

Twenty-one wild birds (n = 17) and mammals (n = 4) were determined to have a died, or suspected to have died, from acute coagulopathy due to anticoagulant rodenticide toxicosis (Table 14).

Thirty-one wild birds (n = 9: included < 1 yr old and 1.5 yr old) and mammals (n = 22: included <1 yr old) in calendar year 2022 had exposure to one or more anticoagulant rodenticide(s) (Table 15). Age and age classes were determined based on plumage and/or the presence of a bursa (for avians), dentition (mammals), and date of death since most species have reproductive seasons in which they predictively mate and produce offspring.


Great horned owl | Tom Koerner, USFWS


Bobcat | Grayson Smith, USFWS


Red-tailed hawk | Mark Bohn, USFWS

Table 14. Summary of cases of anticoagulant rodenticide (AR) toxicosis in non-target wildlife since the implementation of AB1788 on January 1, 2021. Livers from necropsied wildlife were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, rodenticide residues were detected in the liver, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition. Therefore, these diagnoses are reported as "undetermined" toxicosis.

SGAR = second generation anticoagulant rodenticide, FGAR = first generation anticoagulant rodenticide

Date of Death	Species	County	Sex	Age Class	AR Toxicosis	No. SGARs Detected	No. FGARS Detected
AVIAN SUBMISSIONS							
1/18/2022	Red-tailed hawk	Santa Clara	F	Juvenile	Yes	1	0
1/20/2022	Great horned owl	Marin	F	Adult	Yes	3	1
2/3/2022	Barn owl	Ventura	M	Adult	Suspect	3	0
2/14/2022	Red-shouldered hawk	Ventura	M	Adult	Yes	2	0
4/4/2022	Great horned owl	Marin	M	Adult	Yes	3	1
2/10/2022	Great horned owl	Santa Cruz	F	Adult	Yes	3	1
3/31/2022	Great horned owl	Los Angeles	M	Juvenile	Yes	0	1
7/25/2022	Great horned owl	Los Angeles	F	Adult	Yes	3	2
7/26/2022	Great horned owl	Los Angeles	M	Adult	Yes	2	1
7/20/2022	Red-shouldered hawk	Sonoma	F	Adult	Yes	2	0
10/2/2022	Great horned owl	Los Angeles	M	Juvenile	Yes	3	2
10/5/2022	Great horned owl	Contra Costa	M	Juvenile	Yes	3	1
10/21/2022	Great horned owl	San Diego	M	Juvenile	Yes	2	1
4/25/2022	Great horned owl	San Bernardino	F	Adult	Suspect	1	0
11/14/2022	Great horned owl	Santa Clara	F	Adult	Yes	3	1
12/13/2022	Barn owl	San Mateo	F	Juvenile	Yes	2	1
11/15/2022	Great horned owl	Kern	M	Juvenile	Yes	3	0
MAMMAL SUBMISSIONS							
1/6/2022	Coyote	Santa Clara	F	Adult	Yes	2	3
2/10/2022	Gray Fox	Sacramento	M	Adult	Suspect	1	1
10/30/2022	Mountain Lion	Not specified	M	Cub	Suspect	3	1
12/16/2022	Gray Fox	Monterey	M	Adult	Suspect	0	1

Table 15. Summary of cases of anticoagulant rodenticide (AR) exposure in non-target wildlife born or hatched after the implementation of AB1788 on January 1, 2021. Age classes were determined based on plumage, dentition, and reproductive phenology of the species. Livers from necropsied wildlife were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. In some cases, rodenticide residues were detected in the liver, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition. Therefore, these diagnoses are reported as "undetermined" toxicosis.

SGAR = second generation anticoagulant rodenticide, FGAR = first generation anticoagulant rodenticide

Date of Death	Species	County	Sex	Age Class	AR Toxicosis	No. SGARs Detected	No. FGARS Detected
AVIAN SUBMISSIONS							
1/14/2022	Golden eagle	San Luis Obispo	M	Juvenile	No	0	1
1/18/2022	Red-tailed hawk	Santa Clara	F	Juvenile	Yes	1	0
3/31/2022	Great horned owl	Los Angeles	M	Juvenile	Yes	0	1
4/26/2022	Great horned owl	Ventura	F	Juvenile	No	0	0
5/9/2022	Great horned owl	Sonoma	M	Juvenile	No	0	0
10/2/2022	Great horned owl	Los Angeles	M	Juvenile	Yes	3	2
10/5/2022	Great horned owl	Contra Costa	M	Juvenile	Yes	3	1
10/21/2022	Great horned owl	San Diego	M	Juvenile	Yes	2	1
11/16/2022	Great horned owl	Santa Clara	M	Juvenile	No	1	0
12/13/2022	Barn owl	San Mateo	F	Juvenile	Yes	2	1
11/15/2022	Great horned owl	Kern	M	Juvenile	Yes	3	0
MAMMAL SUBMISSIONS							
8/30/2022	Black bear	San Bernardino	Male	1st Year	No	0	1
8/30/2022	Black bear	San Bernardino	Male	1st Year	No	1	1
10/4/2022	Black bear	El Dorado	Female	1st Year	No	1	1
11/10/2022	Black bear	Ventura	Male	1st Year	No	1	0
11/21/2022	Black bear	El Dorado	Male	1st Year	No	1	0
1/20/2022	Coyote	Orange	M	Juvenile	No	3	1
Found 2022	Mountain lion	El Dorado	M	Juvenile	No	0	1
1/19/2022	Coyote	Mono	F	Yearling	No	3	1
2/19/2022	Bobcat	Monterey	F	Juvenile	No	0	1
3/4/2022	Striped skunk	San Francisco	F	Juvenile	No	2	0
3/22/2022	Bobcat	San Mateo	F	Yearling	No	2	1
5/25/2022	Red fox	Contra Costa	M	Pup	No	0	1
7/5/2022	Mountain lion	Nevada	F	Yearling	No	2	1
8/4/2022	Gray fox	Contra Costa	F	Juvenile	No	1	3
9/4/2022	Bobcat	Placer	M	Yearling	No	3	2
10/3/2022	Striped skunk	Plumas	M	Juvenile	No	0	1
10/12/2022	Mountain lion	Orange	F	Cub	No	2	2
10/30/2022	Mountain lion	Not specified	M	Cub	Suspect	3	1
10/18/2022	Mountain lion	Sonoma	M	Cub	No	2	1
11/30/2022	Raccoon	Sonoma	F	Juvenile	No	0	0
12/19/2022	Gray fox	Shasta	F	Juvenile	No	0	1
12/26/2022	San Joaquin kit fox	Kern	F	Juvenile	No	1	1

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Pesticide Exposures & Mortalities in Non-target Wildlife

CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

2023 Annual Report
Wildlife Health Laboratory

2023 Summary of Pesticide Exposures & Mortalities in Non-target Wildlife

By Ryan Bourbour, Krysta Rogers, Nicholas Shirkey, Brandon Munk, Deana Clifford

With contributions from The Wildlife Health Lab staff

CDFW's Canebrake Ecological Reserve. Photo: Ryan Bourbour, CDFW

PREPARED BY THE WILDLIFE HEALTH LABORAOTRY OF THE CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

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INTRODUCTION

The mission of the California Department of Fish and Wildlife (CDFW) is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. As such, a memorandum of understanding (MOU) was developed between the California Department of Pesticide Regulation (CDPR), the County Agriculture Commissioners (CAC), and the CDFW. The purpose of the memorandum is to ensure that pesticides registered in the state of California are used in a manner that protects non-target fish and wildlife resources, while recognizing the need for responsible pest control.

In partial fulfillment of the MOU, this 2023 annual report summarizes documented pesticide exposure and toxicosis in California's fish and wildlife for the respective authorities of CDPR, CAC, and CDFW. These data represent a minimum number of reports for tested animals that died within the reported calendar year and are subject to change as new information becomes available.

DATA COLLECTION & ANALYSIS

The Wildlife Health Laboratory (WHL, formerly the Wildlife Investigations Laboratory) was established in 1941 and is mandated by Fish and Game Code Section 1008 to investigate all diseases and problems relating to wildlife. The WHL has accomplished this goal through collaboration with the public and various organizations to record, collect, and submit wildlife mortalities of interest to the WHL for examination and further diagnostics as needed. The WHL continues communication with interested parties as new information is discovered to aid further cooperation in the goal of maintaining healthy wildlife populations throughout California.

Programmatically the WHL is divided into three units which address health issues: 1) avian, 2) big game, 3) small game and non-game species. The avian unit oversees nearly 600 avian species including non-game (e.g., songbirds, raptors, shorebirds, waders, and seabirds) and game species (e.g., doves, pigeons, quail, turkey, and waterfowl). The big game unit primarily oversees black bear, bighorn sheep, deer, elk, pronghorn, and wild pig with shared responsibility of small game such as tree squirrels, rabbits, and hares. In addition to sharing health surveillance responsibilities with the big game unit, the non-game unit also oversees native non-game mammals, fur bearers, reptiles, and amphibians. This includes a consortium of species such as California tiger salamander, western pond turtles, pika, riparian brush rabbits, skunks, raccoons, foxes, bobcats, mountain lions, and gray wolves.

Wildlife Submissions

Wildlife remains are submitted to the WHL in various ways, primarily by the public – either direct submissions of deceased wildlife to the WHL, submission of living or deceased wildlife to wildlife rehabilitation centers ("rehab"), notification of mortalities to CDFW staff and law enforcement, or other government agency reports (e.g., animal control, sheriff, state and federal Department of Agriculture, U.S. Fish and Wildlife Service, the Park Service, etc.). The WHL also collaborates with universities, non-governmental organizations (NGO), and other agencies on statewide population monitoring projects and provides diagnostic support by conducting postmortem examinations. The WHL contracts with the California Animal Health and Food Safety (CAHFS) Laboratory for further disease and toxicology testing.

Postmortem Examination

Postmortem examinations (necropsies) are performed on wildlife remains at the WHL or the CAHFS Laboratory. If remains cannot be examined within 48 hours of collection, they are stored in a -20°C freezer until an examination can be performed. Prior to necropsy, frozen carcasses are thawed at 4°C or room temperature until they are ready for necropsy. Sex, age class, body condition and, when possible, the cause of death is determined. In addition to necropsy, mortality investigations often include microscopic evaluation of tissues (histology) and ancillary disease and toxicology testing. Tissue samples are collected and placed in 10% formalin for histological evaluation and a complimentary set of tissues are archived in -20C° freezers until submitted to the CAHFS Laboratory for analysis.

Carcasses in advanced stages of decomposition and autolysis are necropsied but formalin tissues may not be collected or submitted since autolysis can obscure or destroy microscopic lesions. In these cases, necropsies are performed, and tissue samples are collected for toxicology testing to assess pesticide exposure but not necessarily toxicosis.

Anticoagulant Rodenticides: Anticoagulant rodenticides (ARs) are grouped into two categories: "first generation anticoagulant rodenticides" (FGARs) which include warfarin (war), coumachlor (cou), diphacinone (diph), and chlorophacinone (chl) and the more toxic "second generation anticoagulant rodenticides" (SGARs) which include brodifacoum (brd), bromadiolone (brm), difenacoum (dfn), and difethialone (dif).

***Non-Anticoagulant Rodenticides & Other Pesticides*:** There are several acutely toxic compounds also used to manage rodent and insect pests, such as bromethalin, strychnine, zinc phosphide, cholecalciferol, organophosphates, and carbamates. Like anticoagulant rodenticides, these compounds, or their metabolites, have been documented in non-target wildlife as a form of mortality or exposure.

Appropriate tissue samples (e.g., gastrointestinal contents, adipose, brain, spinal cord, kidney, liver, gills) for requested tests are also submitted to the CAHFS Laboratory for testing.

Exposure & Toxicosis

Pesticides, including ARs, are not always acutely fatal and there is a high degree of variability among species and individuals in their vulnerability. In the absence of a universal threshold residue value that could indicate AR "toxicosis," we must also rely on antemortem and/or postmortem evidence of coagulopathy unrelated to another identifiable cause of hemorrhage (e.g., trauma, disease, infection).

Individuals are considered to have AR "exposure" if their livers had detectable levels of one or more AR residues (regardless of concentration, reported in parts per billion or ppb) and lack antemortem and/or postmortem evidence of coagulopathy.

For non-ARs, diagnosing toxicosis requires the detection of the compound in the appropriate tissue sample or gastrointestinal contents, and antemortem and/or postmortem evidence in the absence of another identifiable cause (e.g., disease, infection, trauma).

In some cases, rodenticide residues are detected in the tissue sample, but postmortem evidence could not confirm or exclude toxicosis due to advanced decomposition which precludes a definitive diagnosis. Therefore, these diagnoses are reported as “suspected” or “undetermined” toxicosis.

It is important to note that exposure in the absence of toxicosis should not be ignored[1]. The uncertainties about the magnitude and drivers of chronic exposure and/or sub-lethal levels of rodenticide exposure demonstrate the need for continued monitoring. Exposure to ARs may predispose wildlife to excessive hemorrhage following an otherwise non-lethal traumatic injury or increase sensitivity to additional exposure(s)[1].

Additionally, it is important to note that the concentration of ARs quantified in tissue samples does not necessarily equate to risk of toxicosis, as even trace levels (quantities detected below the reporting limit) can be associated with signs of coagulopathy and a toxicosis diagnosis.

AVIAN SUMMARY

According to CDFW records at the time of this report, 936 birds were submitted to the WHL for necropsy, and/or disease or toxicology testing in calendar year 2023. The Eurasian strain of highly pathogenic avian influenza H5N1 continued to impact a diversity of wild birds in California, elsewhere in the United States, and globally. Similar to 2022, the demand for avian influenza surveillance testing increased the number of avian submissions to WHL.

Birds were submitted for various reasons by wildlife rehabilitators, members of the public, non-profit organizations, universities, CDFW staff and law enforcement, and other agencies (Table 1). Wildlife rehabilitators made up the majority of submissions, followed by agencies and specifically, CDFW. However, it should be noted that the majority of these reports originated with a member of the public.

Flight and tail feathers of an adult Red-tailed Hawk. Photo: Ryan Bourbour, CDFW

Table 1. Total number of wild bird remains submitted to the Wildlife Health Laboratory for necropsy in 2023 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Birds Submitted
CDFW	143
NGO/Non-Profit	34
Other Government / Military	72
Private Consultant / Energy	26
Public	30
Rehab / Zoo / Sanctuary	627
University	4
Total	**936**

Anticoagulant Rodenticide Exposure & Toxicosis

Of necropsied birds, 42 were tested for anticoagulant rodenticide exposure. Tested birds represent 33% (19/58) of California counties (Table 2). All age classes and sexes were represented in submitted carcasses.

Waterfowl and waterbirds (n = 391) accounted for the largest percentage of birds submitted followed by raptors (n = 340). Raptors were disproportionally screened for exposure to anticoagulant rodenticides given they are more likely to be exposed to one or more analyte(s) through their diet (Table 3). Of the 73.8% (31/42) of birds with exposure, 35.5% (11/31) were cases of raptors diagnosed with anticoagulant rodenticide toxicosis. One common raven screened for anticoagulant rodenticides had exposure (Table 3).

Seventeen of the 31 exposed birds had two or more anticoagulant rodenticides detected in the liver (Figure 1). Prevalence of exposure to second generation anticoagulant rodenticides was 61.9% (26/42) while exposure to first generation anticoagulant rodenticides was 35.7% (15/42). Brodifacoum, bromadiolone, and difethialone were the most common second-generation anticoagulant rodenticides detected in liver samples (Figure 2). Diphacinone and chlorophacinone were the most common first-generation anticoagulant rodenticides detected in liver samples (Figure 2). Diagnoses of anticoagulant toxicosis were associated with varying liver concentration levels including trace (Figure 3; Table 4). Detectable FGAR concentration levels ranged from 96 to 460 ppb with detections of trace levels in 13 liver samples (Table 5). Detectable SGAR concentration levels ranged from 53 to 560 ppb with detections of trace levels in 28 liver samples (Table 5). None of the birds sampled had detectable levels of exposure to warfarin, difenacoum, or coumachlor. Out of the 31 birds exposed to ARs, 45.2% (14/31) were Hatch-Year (<1 year old; Table 6). Out of the Hatch-Year birds that were exposed, 35.7% (5/14) died from AR toxicosis (Table 6)

Other Pesticides

Other pesticide-related investigations included one incident involving common ravens in Mendocino County. Avitrol was detected in a single common raven submitted from Mendocino

County in September 2023 where multiple ravens had been reported dead over several days. The resident who reported the raven that was ultimately submitted for testing observed the raven having seizures before death.

Table 2. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 42 tested wild birds submitted to the Wildlife Health Laboratory in 2023 by county. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety

County	Birds Tested	Birds with Exposure	Exposure Prevalence (%)	Confirmed/ Suspected Toxicosis
Alameda	5	5	100.0	0
Contra Costa	3	2	66.7	1
Del Norte	1	1	100.0	0
Humboldt	3	3	100.0	0
Kern	2	2	100.0	1
Los Angeles	1	1	100.0	1
Modoc	1	0	0.0	0
Monterey	2	1	50.0	0
San Diego	5	5	100.0	3
San Joaquin	1	1	100.0	1
San Luis Obispo	5	3	60.0	1
San Mateo	1	1	100.0	1
Santa Clara	2	2	100.0	1
Shasta	1	0	0.0	0
Siskiyou	1	0	0.0	0
Solano	2	0	0.0	0
Sonoma	1	1	100.0	0
Ventura	4	2	50.0	1
Yolo	1	1	100.0	0
Total	**42**	**31**	**73.8**	**11**

Barn Owl nestlings in an urban nest box in Yolo County. Photo: Ryan Bourbour, CDFW

Table 3. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 42 wild birds submitted to the Wildlife Health Laboratory in 2023 by species (common name).

Species	No. Tested	No. Birds with Exposure	Exposure Prevalence (%)	No. Confirmed/ Suspected Toxicosis
Bald Eagle	1	0	0.0	0
Barn Owl	8	4	50.0	3
Common Raven	1	1	100.0	0
Cooper's Hawk	1	0	0.0	0
Golden Eagle	5	4	80.0	0
Great Horned Owl	11	10	90.9	4
Red-shouldered Hawk	1	1	100.0	0
Red-tailed Hawk	11	8	72.7	4
Turkey Vulture	3	3	100.0	0
Total	**42**	**31**	**73.8**	**11**

An adult Great Horned Owl perched on the edge of an orchard in Yolo County. Photo: Ryan Bourbour, CDFW

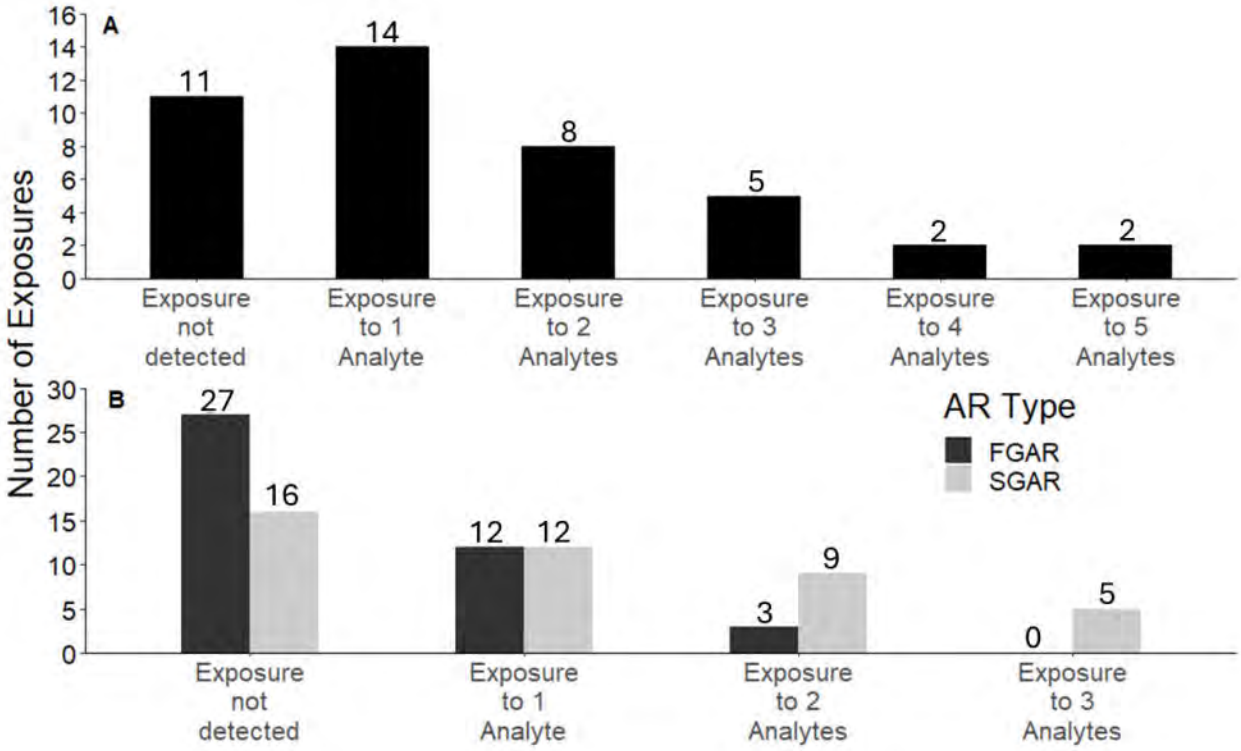

Figure 1. **(A)** Number of anticoagulant rodenticide residues detected in the livers of 31 wild birds in 2023. **(B)** Number of anticoagulant residues detected in the livers of 31 wild birds separated by SGAR and FGAR.

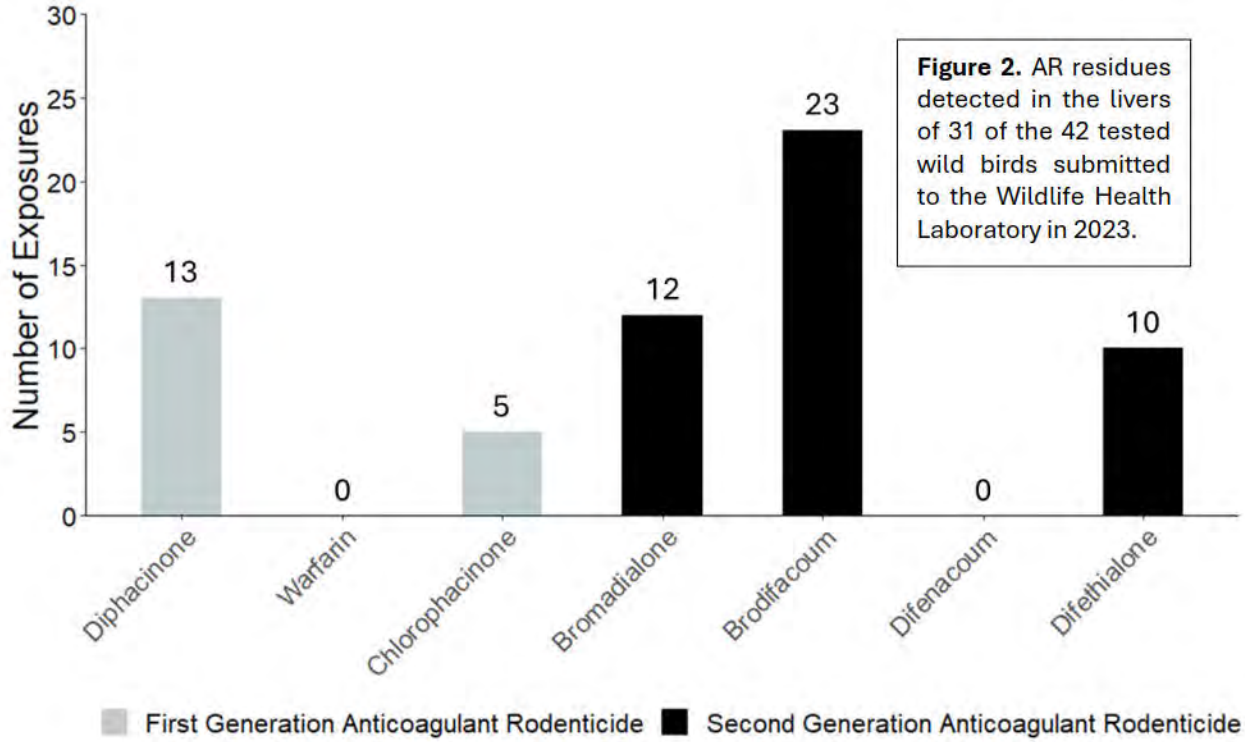

Figure 2. AR residues detected in the livers of 31 of the 42 tested wild birds submitted to the Wildlife Health Laboratory in 2023.

Table 4. AR exposure in the 11 out of 31 individual birds that had evidence supporting a diagnosis for AR toxicosis in 2023. Note that toxicosis can occur at varying levels of AR concentrations for all analytes detected, including trace levels.

Species	Brm (ppb)	Brd (ppb)	Dif (ppb)	Chl (ppb)	Diph (ppb)	Final Diagnosis
Barn Owl	180	**Trace**	—	**Trace**	—	AR toxicosis
Barn Owl	63	240	**Trace**	—	**Trace**	AR toxicosis
Barn Owl	57	100	68	**Trace**	**Trace**	AR toxicosis
Great Horned Owl	**Trace**	140	**Trace**	—	—	AR toxicosis
Great Horned Owl	180	54	**Trace**	**Trace**	**Trace**	AR toxicosis
Great Horned Owl	—	—	—	—	96	AR toxicosis
Great Horned Owl	—	—	130	—	460	AR toxicosis
Red-tailed Hawk	**Trace**	**Trace**	**Trace**	**Trace**	—	AR toxicosis
Red-tailed Hawk	—	53	**Trace**	—	—	AR toxicosis suspect
Red-tailed Hawk	—	—	—	—	120	AR toxicosis
Red-tailed Hawk	—	560	—	—	—	AR toxicosis

A juvenile Red-tailed Hawk at Ash Creek Wildlife Area. Photo: Ryan Bourbour, CDFW

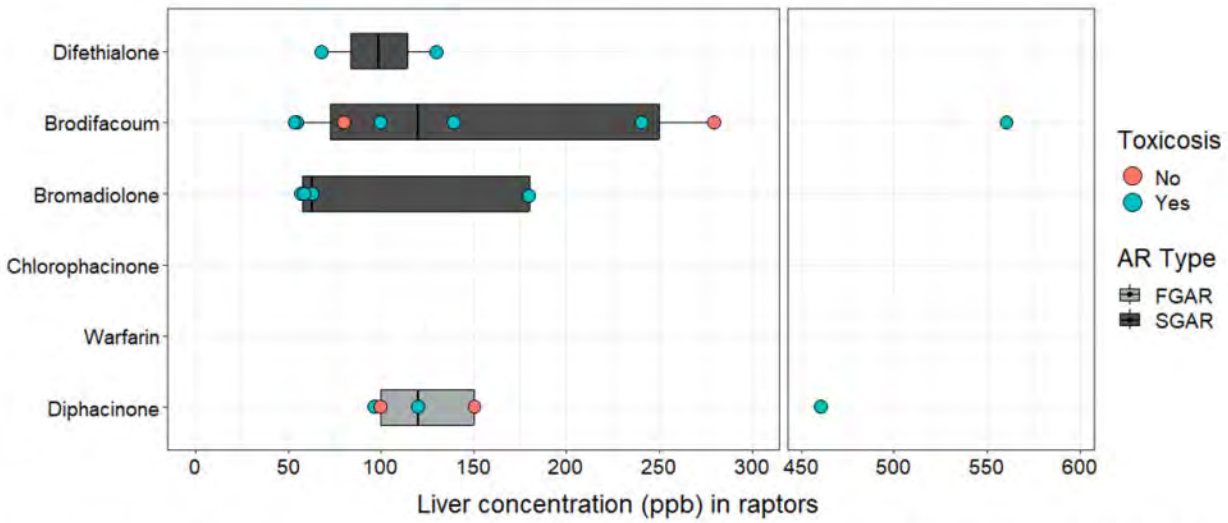

Figure 3. Boxplot to visualize AR concentrations (ppb) quantitated in the livers of 20 of the 31 wild birds that tested positive for AR exposure in 2023. Colored marks indicate concentrations whether or not toxicosis was confirmed. Note that toxicosis can occur at varying levels of AR concentrations for all analytes detected, including trace levels (Table 5). Box plot summary can be found in Appendix 1.1.

Table 5. Summary of AR liver concentration (ppb) levels detected in the 31 of 42 wild birds that tested positive for AR exposure in 2023. Summary includes concentration mean and standard error (SE) of the mean, range, and number of trace detections.

AR Type	Analyte	Mean ± SE (ppb)	Range (ppb)	No. of Trace Detections
FGAR	**Diphacinone**	185.2 ± 69.4	96 – 460	8
	Chlorophacinone	—	—	5
SGAR	**Bromadiolone**	107.6 ± 29.6	57 – 180	7
	Brodifacoum	188.3 ± 60.9	53 – 560	13
	Difethialone	99 ± 31.0	68 – 130	8

An immature Red-tailed Hawk hunting on a Yolo County farm field. Photo: Ryan Bourbour, CDFW

Table 6. FGAR and SGAR exposures in 31 wild birds submitted to the Wildlife Health Laboratory in 2023 by species, county, sex, and age, and cause of death. Birds aged as Hatch-Year (HY) and Second-Year (SY) with SGAR detections are confirmed exposures after the implementation of AB1788's restrictions on SGAR-use in California. Note: HY birds are <1 year old, SY birds are 1-2 years old.

Species	County	Sex	Age Category	No. of FGARs	No. of SGARs	Cause of Death
Barn Owl	San Mateo	F	**HY**	**1**	**2**	**AR toxicosis**
Barn Owl	San Diego	F	Adult	0	2	Trauma
Barn Owl	San Diego	M	Adult	**1**	**3**	**AR toxicosis**
Barn Owl	San Diego	F	Adult	**2**	**3**	**AR toxicosis**
Common Raven	Humboldt	M	Adult	0	1	Trauma
Great Horned Owl	Kern	M	**HY**	0	3	**AR toxicosis**
Great Horned Owl	Humboldt	M	Adult	0	2	Nutritional
Great Horned Owl	Kern	M	Adult	0	2	Nutritional
Great Horned Owl	Los Angeles	M	Adult	**2**	**3**	**AR Toxicosis**
Great Horned Owl	Humboldt	F	Adult	**1**	**2**	Trauma
Great Horned Owl	Alameda	F	**HY**	0	1	Trauma
Great Horned Owl	San Joaquin	M	**HY**	1	0	**AR Toxicosis**
Great Horned Owl	Monterey	F	**HY**	**1**	**2**	Trauma
Great Horned Owl	Ventura	M	Adult	0	1	Trauma
Great Horned Owl	San Diego	F	**HY**	**1**	**1**	**AR Toxicosis**
Golden Eagle	Alameda	F	Adult	0	2	Disease
Golden Eagle	San Diego	M	**HY**	1	0	Trauma
Golden Eagle	Alameda	F	**SY**	1	0	Trauma
Golden Eagle	San Luis Obispo	M	**HY**	**2**	**1**	Trauma
Red-shouldered Hawk	San Luis Obispo	M	Adult	0	1	Trauma
Red-tailed Hawk	Alameda	M	Adult	0	2	Trauma
Red-tailed Hawk	Del Norte	M	Adult	0	1	Trauma
Red-tailed Hawk	Contra Costa	F	Adult	**1**	**3**	**AR toxicosis**
Red-tailed Hawk	Ventura	F	Adult	0	2	**Suspect AR Toxicosis**
Red-tailed Hawk	Santa Clara	F	**HY**	1	0	**AR toxicosis**
Red-tailed Hawk	Contra Costa	M	**HY**	0	1	Nutritional
Red-tailed Hawk	San Luis Obispo	M	Adult	0	1	**AR toxicosis**
Red-tailed Hawk	Yolo	F	**HY**	0	1	Undetermined
Turkey Vulture	Alameda	M	**HY**	1	0	Trauma
Turkey Vulture	Santa Clara	F	**HY**	**1**	**1**	Trauma
Turkey Vulture	Sonoma	F	**HY**	0	1	Trauma

American Black Bear in Humboldt County. Photo: CDFW Science Institute & Lands Program

BIG GAME SUMMARY

The remains and/or tissues of 142 big game mammals were submitted to the WHL for necropsy and/or toxicology testing in the year 2023.

Approximately 92% (130/142) of the big game carcasses were submitted by the CDFW and other agencies (Table 7). However, it should be noted that public reports represent the original source for most CDFW submissions.

Table 7. Total number of wild big game mammal tissues or remains submitted to the Wildlife Health Laboratory in 2023 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Big Game Mammals Submitted
CDFW	130
Other Government Agency	1
Public	6
Rehab	5
Total	**142**

Anticoagulant Rodenticide Exposure

Of necropsied big game mammals, 16 were tested for AR exposure. Big game mammals were submitted from 11 of the 58 counties in California (Table 8). All age classes and sexes were represented in submitted carcasses.

Of the 16 big game animals tested, black bears accounted for 15 (93.8%) of the animals tested. Six of the 15 black bears (40%) tested positive for AR exposure (Table 9). Four of the 15 (26.7%) black bears tested positive for one AR and 2 of the 15 (13.3%) tested positive for two ARs regardless of first- or second generation (Figure 4).

Of the 6 black bears that tested positive for ARs, 5 were positive for SGARs: brodifacoum (n=5) and difethialone (n=1). Two black bears tested positive for the FGAR diphacinone, one bear at trace levels and another bear with 1200 ppb in liver tissue (Table 9). Detectable SGAR concentration levels ranged from 99 to 630 ppb with detections of trace levels in 3 bears (Table 10).

Brodifacoum was the most common analyte detected in tested liver samples (Figure 5). Warfarin, chlorophacinone, coumachlor, bromadiolone and difenacoum were not detected in any of the submitted liver samples.

None of the 16 exposures resulted in cases of anticoagulant rodenticide toxicosis.

Bromethalin Exposure & Other Pesticides

Adipose, brain, or liver tissue from 13 black bears from 11 California counties were tested for exposure to the neurotoxic rodenticide, bromethalin (Table 10). Of the four cases where bromethalin was detected, concurrent exposure to ARs was also detected in two bears (Table 12). One bromethalin positive bear tested positive for diphacinone (trace levels), and the second bromethalin positive bear tested positive for diphacinone (1200 ppb) and brodifacoum (trace levels). Acetylcholinesterase activity was measured as within normal limits for one bear from Sierra County.

Black-tailed Deer at Upper Butte Wildlife Area. Photo: Ryan Bourbour, CDFW

Table 8. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 16 tested wild big game mammals submitted to the Wildlife Health Laboratory in 2023 by county.

County	No. Tested	No. Exposed	%Exposed	No. Confirmed Toxicosis
Butte	2	0	0	0
Del Norte	1	0	0	0
El Dorado	3	1	33.3	0
Humboldt	1	0	0	0
Los Angeles	1	1	100	0
Placer	2	2	100	0
San Bernardino	1	1	100	0
Sierra	1	1	100	0
Siskiyou	2	0	0	0
Sonoma	1	0	0	0
Tuolumne	1	0	0	0
Total	**16**	**6**	**37.5**	**0**

Table 9. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 16 wild big game mammals submitted to the Wildlife Health Laboratory in 2023 by species.

Big Game Species	No. Tested	No. Exposed	%Exposed	No. Confirmed Toxicosis
Black Bear	15	6	40	0
Black Tailed Deer/ Mule Deer	1	0	0	0
Total	**16**	**6**	**37.5**	**0**

American Black Bear with cubs at Hallelujah Junction Wildlife Area. Photo: CDFW Science Institute & Lands Program

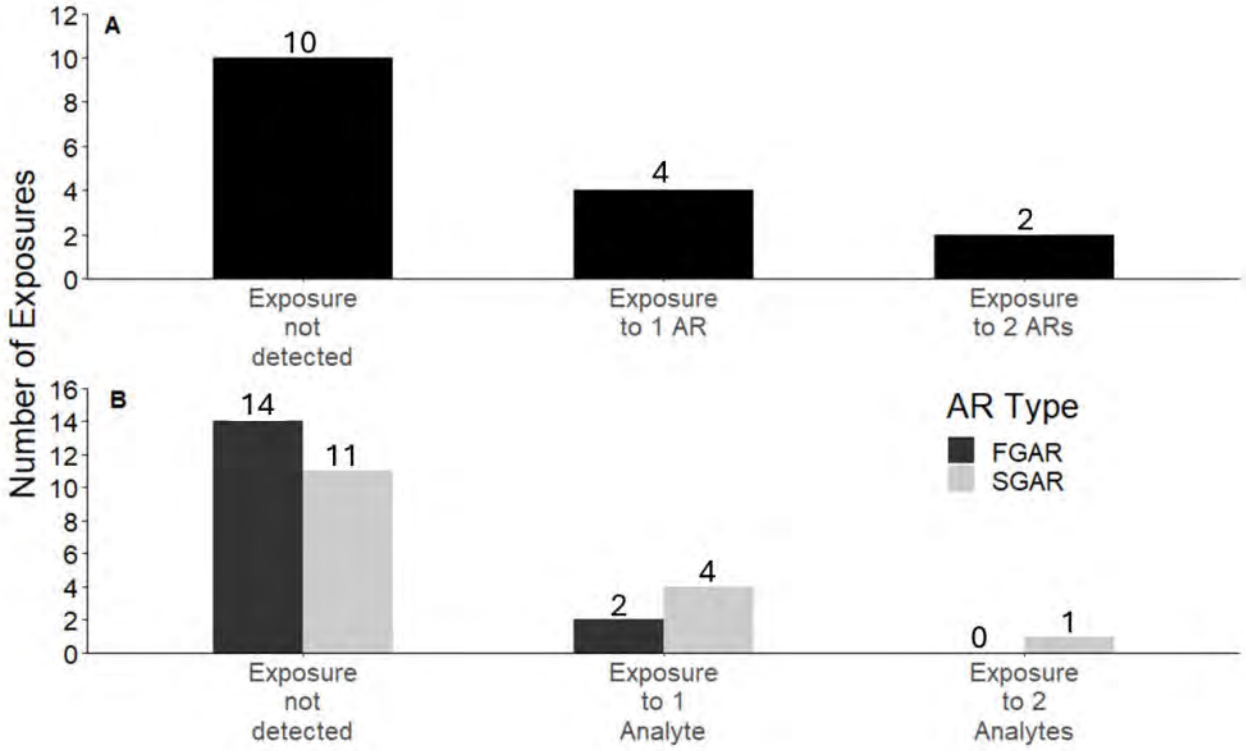

Figure 4. (A) Number of AR residues detected in the livers of 16 wild big game mammals in 2023. **(B)** Number of AR residues detected in the livers of 16 wild big game mammals separated by FGAR and SGAR in 2023. After postmortem examination, livers were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Table 10. Summary of AR liver concentration (ppb) levels detected in the 6 black bears that tested positive for AR exposure in 2023.

AR Type	Analyte	Liver concentration (ppb)	No. of Trace Detections
FGAR	**Diphacinone**	1200	1
SGAR	**Brodifacoum**	150, 99	3
	Difethialone	630	0

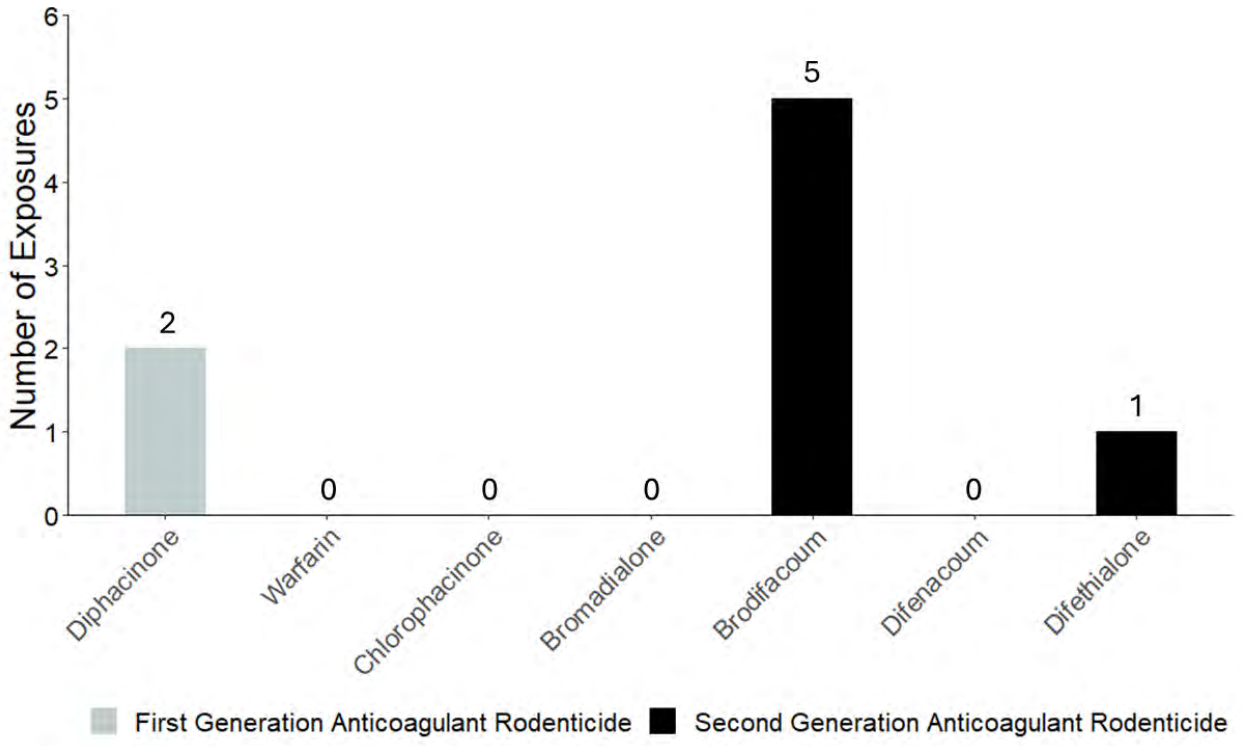

Figure 5: Anticoagulant rodenticide residues detected in the livers of 6 of the 16 tested wild big game mammals submitted to the Wildlife Health Laboratory in 2023.

Pronghorn at Great Basin Springs. Photo: Ryan Bourbour, CDFW

Table 11. Bromethalin exposure in 13 wild black bears submitted to the Wildlife Health Laboratory in 2023 by county. Adipose, brain, or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. *Suspicion for bromethalin toxicosis was raised for one bear in Placer County; however, lesions in the brain tissue could not confidently be distinguished from freeze-thaw artifacts.

County	No. Tested	No. Exposed	%Exposed	No. Suspected Toxicosis
Butte	2	0	0	0
El Dorado	3	1	33.3	0
Humboldt	1	0	0	0
Los Angeles	1	0	0	0
Placer	2	1	50	0*
San Bernardino	1	1	100	0
Sierra	1	0	0	0
Siskiyou	1	1	100	0
Sonoma	1	0	0	0
Total	**13**	**4**	**30.8**	**0**

Table 12. AR and bromethalin exposure in 8 wild black bears submitted to the Wildlife Health Laboratory in 2023. Adipose, brain, or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA. *Suspicion for bromethalin toxicosis was raised for one bear in Placer County; however, lesions in the brain tissue could not confidently be distinguished from freeze-thaw artifacts.

County	Sex	Age Category	FGAR Exposure	SGAR Exposure	Bromethalin Exposure
El Dorado	Male	2nd Year	—	—	**Yes**
El Dorado	Female	Adult	—	Yes	—
Los Angeles	Male	Sub-adult	—	Yes	—
Placer	Male	2nd Year	**Yes**	—	**Yes***
Placer	Male	Adult	—	Yes	—
San Bernardino	Female	2nd Year	**Yes**	**Yes**	**Yes**
Sierra	Female	Adult	—	Yes	—
Siskiyou	Male	2nd Year	—	—	Yes

Coyotes at Hallelujah Junction Wildlife Area. Photo: CDFW Science Institute & Lands Program

SMALL GAME & NON-GAME SUMMARY

The remains of 172 small- and non-game wildlife were submitted to the WHL for necropsy in 2023. Small game and non-game animals were submitted for various reasons by wildlife rehabilitators, members of the public, non-profit organizations, universities, CDFW staff and law enforcement, and other agencies. Wildlife rehabilitators made up 21% (36/172) of submissions, followed by 48% (82/172) submissions from CDFW (Table 13). Toxicology testing was not performed on the herptiles in 2023. Therefore, the remainder of this section will address completed test results for mammals.

Table 13. Total number of wild small- and non-game animal tissues or remains submitted to the Wildlife Health Laboratory in 2023 based on the primary submitter's affiliation. Many submissions that are non-public originated as a public report.

Submitter Affiliation	No. Small and Non-game Animals Submitted
Animal Control	5
CDFW	82
NGO/Non-Profit	4
Other	2
Other Government Agency	21
Private Biological Consultant	2
Public	12
Rehab/Zoo/Sanctuary	36
University Affiliate	8
Total	**172**

Anticoagulant Rodenticide Exposure

Of necropsied small- and non-game wildlife, 70 were tested for pesticide exposure. Sampled remains with final reports represented 53.4% (31/58) of California counties (Table 14). All age classes and sexes were represented.

Mountain lions accounted for the largest percentage of mammal samples submitted to the WHL (Table 15). In total, 78.6% (55/70) of mammals tested had exposure to one or more anticoagulant rodenticide and 54% (38/70) of the tested animals had exposure to two or more anticoagulant rodenticides regardless of first- or second generation (Figure 9). Three mountain lions from Placer, Santa Cruz, and Ventura counties tested positive for five different anticoagulant rodenticides. Five anticoagulant rodenticides were also detected in one bobcat from El Dorado County, one gray fox from Santa Clara County, and one San Joaquin kit fox from Kern County. None of the 56 exposures in 2023 were confirmed anticoagulant rodenticide toxicosis (Table 15).

Brodifacoum, bromadiolone, and diphacinone were the most common analytes detected in liver samples (Figure 10). Analytes detected in liver tissues were quantitated at a wide range of concentrations, including trace levels (Figure 11; Table 16). None of the tested samples in 2023 had detectable levels of exposure to coumachlor or difenacoum.

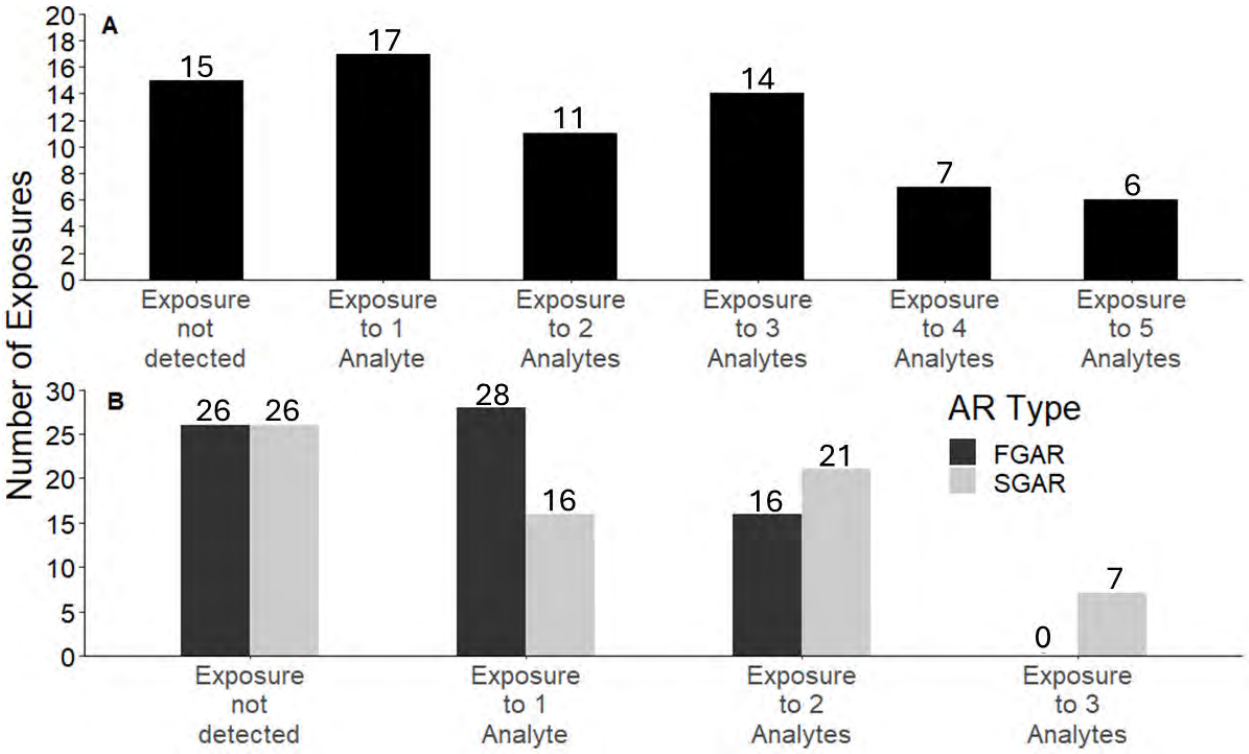

Figure 9. **(A)** Number of anticoagulant rodenticide residues detected in the livers of 70 wild non-game mammals in 2023. **(B)** Number of anticoagulant residues detected in the livers of 70 wild non-game mammals separated by first (FGAR) and second (SGAR) generation anticoagulant rodenticides in 2023.

Mountain Lion at Burton Mesa Ecological Reserve. Photo: CDFW Science Institute & Lands Program

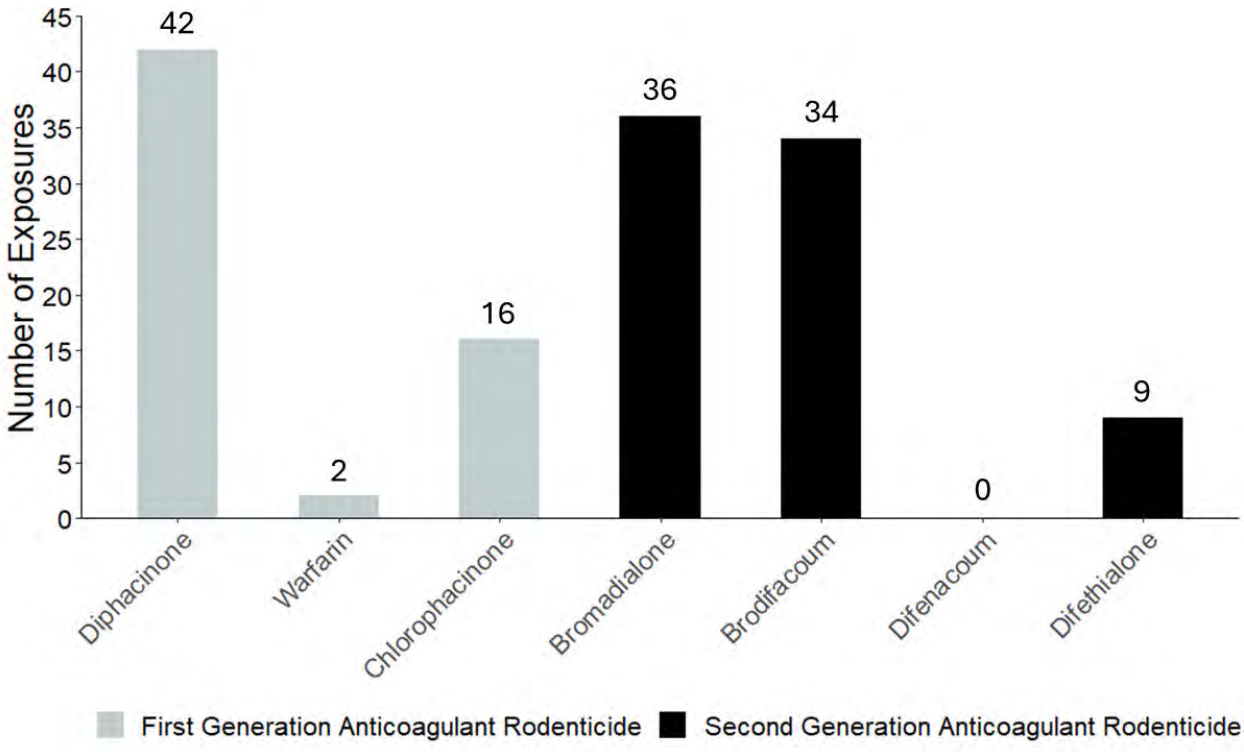

Figure 10. AR residues detected in the livers of 55 of the 70 tested wild non-game mammals submitted to the Wildlife Health Laboratory in 2023. Each bar displays number of exposures at the top.

Table 14. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 70 tested wild non-game animals submitted to the Wildlife Health Laboratory in 2023 by California county.

County	No. Non-game Tested	No. Non-game Exposed	Exposure Prevalence (%)	No. Confirmed or Suspected Toxicosis
Alameda	1	1	100.0	0
Butte	5	3	60.0	0
Calaveras	1	1	100.0	0
Contra Costa	1	0	0.0	0
El Dorado	3	3	100.0	0
Humboldt	1	1	100.0	0
Inyo	3	2	66.7	0
Kern	6	6	100.0	0
Lake	1	1	100.0	0
Lassen	2	1	50.0	0
Mariposa	2	1	50.0	0
Mendocino	2	1	50.0	0
Modoc	5	3	60.0	0
Mono	3	3	100.0	0
Napa	1	1	100.0	0
Placer	1	1	100.0	0
Plumas	2	1	50.0	0
Riverside	1	1	100.0	0
Sacramento	1	1	100.0	0
San Bernardino	3	2	66.7	0
San Diego	5	4	80.0	0
San Joaquin	1	1	100.0	0
San Mateo	1	1	100.0	0
Santa Clara	2	1	50.0	0
Santa Cruz	3	3	100.0	0
Shasta	1	1	100.0	0
Siskiyou	3	2	66.7	0
Sonoma	5	5	100.0	0
Tehama	1	1	100.0	0
Ventura	1	1	100.0	0
Yuba	2	1	50.0	0
Total	**70**	**55**	**78.6**	**0**

Table 15. Exposure prevalence and number of confirmed toxicosis cases of anticoagulant rodenticides in 70 wild non-game mammals submitted to the Wildlife Health Laboratory in 2023 by species (common name).

Species	No. Non-game Tested	No. Non-game Exposed	Exposure Prevalence (%)	No. Confirmed or Suspected Toxicosis
Beaver	1	0	0.0	0
Bobcat	13	11	84.6	0
Coyote	2	2	100.0	0
Desert Cottontail	1	0	0.0	0
Eastern Gray Squirrel	1	0	0.0	0
Fisher	3	1	33.3	0
Gray Fox	10	9	90.0	0
Gray Wolf	2	0	0.0	0
Mountain Lion	28	26	92.8	0
Porcupine	1	0	0.0	0
Raccoon	2	2	100.0	0
San Joaquin Kit Fox	3	3	100.0	0
Striped Skunk	3	1	33.3	0
Total	**70**	**55**	**78.6**	**0**

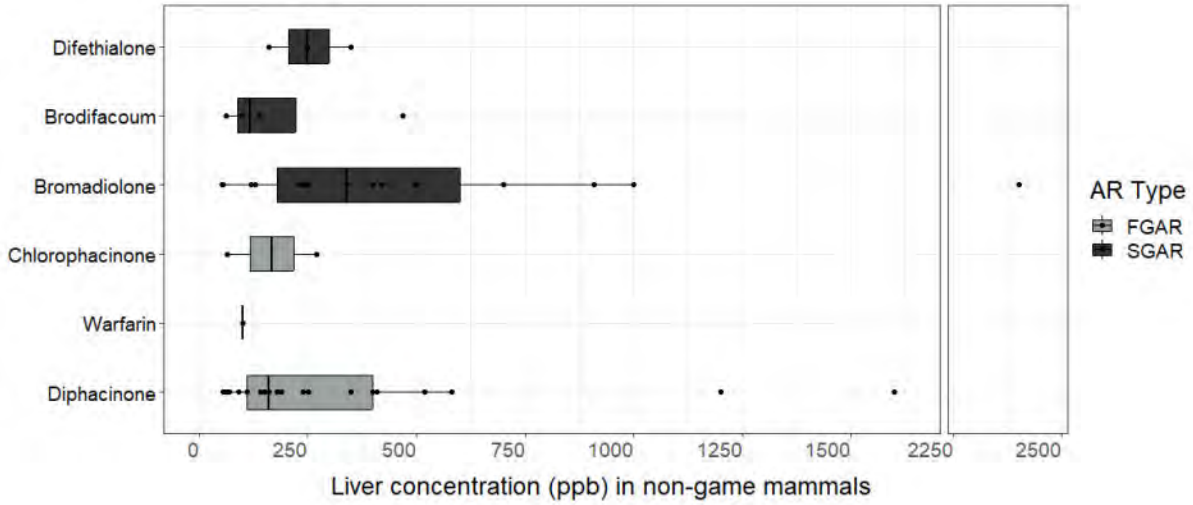

Figure 11. Boxplot to visualize AR concentrations (ppb) in the livers of 50 of the 70 tested wild non-game mammals submitted to the Wildlife Health Laboratory in 2023 where detectable levels were quantitated. This figure does not include instances of trace level detections (see Table 14). Box plot summary can be found in Appendix 1.2.

Table 16. Anticoagulant rodenticide concentrations (ppb) and number of trace detections in the livers of wild non-game mammals submitted to the Wildlife Health Laboratory in 2023. Summary includes concentration mean and standard error (SE) of the mean, range, and number of trace detections.

AR Type	Analyte	Mean ± SE (ppb)	Range (ppb)	Trace Level Detections
FGAR	Diphacinone	308.5 ± 73.0	56 – 1600	18
	Warfarin	100	100	1
	Chlorophacinone	167.5 ± 102.5	65 – 270	14
SGAR	Bromadiolone	516.5 ± 154.1	52 – 2400	21
	Brodifacoum	192.5 ± 93.8	63 – 670	30
	Difethialone	253.3 ± 54.9	160 – 350	6

Bromethalin Exposure

Adipose or brain from 62 animals across 28 counties was tested for exposure to the neurotoxic rodenticide, bromethalin (Table 17). Nine of the tested animals (14.5%) had exposure to bromethalin. These exposures resulted in one case each of confirmed toxicosis and suspected toxicosis (Table 17; Table 18). The case of confirmed bromethalin toxicosis was a gray fox in Mendicino County observed with neurological symptoms before death and tested positive for the bromethalin metabolite in adipose tissue, stomach contents, and feces. In the suspected bromethalin toxicosis case, a raccoon in good nutritional condition from Sonoma County showed neurological symptoms and tested positive for bromethalin in brain tissue, but also had trace exposure to chlorophacinone with some signs of hemorrhaging. With the raccoon testing negative for diseases that could cause neurological signs, this case was classified as suspected toxicosis.

Of the nine non-game wildlife that tested positive for bromethalin, six were concurrently tested for anticoagulant rodenticide exposure. Five out of six (83.3%) non-target wildlife were concurrently exposed to bromethalin and to one or more anticoagulant rodenticides. Anticoagulant rodenticides were not detected in one gray fox from Mendicino County; however, this mortality was confirmed bromethalin toxicosis. Concurrent exposures for all ages are summarized in Tables 19 and 20.

Gray Foxes at Sycuan Peak Ecological Reserve. Photo: CDFW Science Institute & Lands Program.

Table 17. Bromethalin exposure in 62 wild non-game mammals submitted to the Wildlife Health Laboratory in 2023 by county. Adipose, brain, or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

County	No. Non-game Tested	No. Non-game Exposed	Exposure Prevalence (%)	No. Confirmed or Suspected Toxicosis
Butte	6	1	16.7	0
Calaveras	1	0	0.0	0
Contra Costa	1	0	0.0	0
El Dorado	3	0	0.0	0
Inyo	3	0	0.0	0
Kern	5	0	0.0	0
Lake	1	0	0.0	0
Lassen	2	0	0.0	0
Mariposa	1	0	0.0	0
Mendocino	2	1	50.0	1
Modoc	4	0	0.0	0
Mono	2	0	0.0	0
Napa	1	0	0.0	0
Nevada	2	1	50.0	0
Placer	2	0	0.0	0
Plumas	2	0	0.0	0
Sacramento	1	0	0.0	0
San Bernardino	2	0	0.0	0
San Diego	4	0	0.0	0
San Joaquin	1	0	0.0	0
San Mateo	1	0	0.0	0
Santa Clara	1	1	100.0	0
Santa Cruz	2	0	0.0	0
Siskiyou	3	1	33.3	0
Sonoma	6	4	66.7	1
Tuolumne	1	0	0.0	0
Ventura	1	0	0.0	0
Yuba	1	0	0.0	0
Grand Total	**62**	**9**	**14.5**	**2**

Table 18. Bromethalin exposure in 62 wild non-game mammals submitted to the Wildlife Health Laboratory in 2023 by county. Adipose, brain, or liver were submitted for toxicology testing to the California Animal Health and Food Safety Laboratory in Davis, CA.

Species	No. Non-game Tested	No. Non-game Exposed	Exposure Prevalence (%)	No. Confirmed or Suspected Toxicosis
Bobcat	5	0	0.0	0
Coyote	2	0	0.0	0
Desert Cottontail	1	0	0.0	0
Fisher	2	0	0.0	0
Gray Fox	13	5	38.5	1
Gray Wolf	2	0	0.0	0
Mountain Lion	23	1	4.3	0
Opossum	1	0	0.0	0
Porcupine	1	0	0.0	0
Raccoon	2	1	50.0	1
San Joaquin Kit Fox	4	0	0.0	0
Striped Skunk	6	2	33.3	0
Total	**62**	**9**	**14.5**	**2**

Bobcat at Camp Cady Wildlife Area. Photo: CDFW Science Institute & Lands Program

Table 19. Concurrent anticoagulant rodenticide and bromethalin exposure in 20 young non-game mammals submitted to the Wildlife Health Laboratory in 2023. Juveniles, pups, and cubs are confirmed exposures that occurred after the implementation of AB1788. *Represents confirmed/suspected bromethalin toxicosis case.

Species	County	Sex	Age Class	No. FGARs	No. SGARs	Bromethalin Exposure
Bobcat	Santa Cruz	F	Sub-adult (1-2 years)	1	0	not tested
Bobcat	Riverside	M	Juvenile (<1 year)	**1**	**1**	not tested
Coyote	San Bernardino	M	Juvenile (<1 year)	1	0	—
Gray Fox	San Diego	M	Pup (<1 month)	**1**	**1**	not tested
Gray Fox	San Joaquin	F	Juvenile (<1 year)	**1**	**1**	—
Gray Fox	Butte	M	Juvenile (<1 year)	1	0	—
Mountain Lion	Lake	M	Sub-adult (<2 years)	**2**	**1**	—
Mountain Lion	Santa Cruz	M	Sub-adult (<2 years)	**2**	**3**	—
Mountain Lion	Plumas	M	Sub-adult (<2 years)	1	0	—
Mountain Lion	Lassen	M	Cub (<1 year)	1	0	—
Mountain Lion	Mono	F	Cub (<1 year)	**1**	**2**	—
Mountain Lion	Mono	F	Sub-adult (<2 years)	**2**	**2**	not tested
Mountain Lion	Shasta	F	Cub (<1 year)	1	0	not tested
Mountain Lion	Mariposa	F	Sub-adult (<2 years)	**1**	**1**	—
Mountain Lion	Tehama	M	Yearling (1 year)	**1**	**2**	not tested
Mountain Lion	Siskiyou	F	Cub (<1 year)	0	1	—
Mountain Lion	Siskiyou	M	Sub-adult (<2 years)	**1**	**0**	**Yes**
Mountain Lion	San Diego	F	Sub-adult (<2 years)	**1**	**1**	—
Raccoon	Sonoma	M	Juvenile (<1 year)	**1**	**0**	**Yes***
Raccoon	El Dorado	M	Juvenile (<1 years)	1	0	—

Coyote at Napa-Sonoma Marshes Wildlife Area. Photo: CDFW Science Institute & Lands Program

Table 20. Concurrent anticoagulant rodenticide and bromethalin exposure in 36 adult non-game mammals submitted to the Wildlife Health Laboratory in 2023. AR exposures may or may not have occurred after the implementation of AB1788. *Represents confirmed bromethalin toxicosis case.

Species	County	Sex	Age Class	No. FGARs	No. SGARs	Bromethalin Exposure
Bobcat	Humboldt	F	Adult	0	1	not tested
Bobcat	El Dorado	M	Adult	**2**	**3**	—
Bobcat	Kern	F	Adult	**1**	**1**	—
Bobcat	Sacramento	M	Adult	0	2	not tested
Bobcat	Kern	F	Adult	**1**	**3**	not tested
Bobcat	Alameda	Unknown	Adult	0	2	not tested
Bobcat	San Bernardino	F	Adult	**2**	**2**	—
Bobcat	San Diego	M	Adult	**2**	**1**	—
Bobcat	Kern	F	Adult	0	1	not tested
Coyote	Butte	M	Adult	**2**	**1**	—
Fisher	Mendocino	M	Adult	**1**	**2**	—
Gray Fox	Sonoma	M	Adult	**1**	**2**	**Yes**
Gray Fox	Sonoma	Unknown	Adult	**1**	**2**	**Yes**
Gray Fox	Napa	F	Adult	0	1	—
Gray Fox	Santa Cruz	M	Adult	0	1	—
Gray Fox	Santa Clara	F	Adult	**2**	**3**	**Yes**
Gray Fox	Calaveras	F	Unknown	1	0	—
Gray Fox	Mendocino	M	Adult	0	0	Yes*
Mountain Lion	Ventura	F	Adult	**2**	**3**	—
Mountain Lion	San Mateo	M	Adult	**1**	**2**	—
Mountain Lion	Butte	M	Adult	**1**	**2**	—
Mountain Lion	Modoc	F	Adult	0	2	—
Mountain Lion	Modoc	M	Adult	**1**	**2**	not tested
Mountain Lion	Placer	F	Adult	**2**	**3**	—
Mountain Lion	El Dorado	F	Adult	**2**	**2**	—
Mountain Lion	Inyo	M	Adult	**2**	**2**	—
Mountain Lion	Mono	M	Adult	**1**	**2**	—
Mountain Lion	Inyo	M	Adult	**1**	**2**	—
Mountain Lion	Sonoma	M	Adult	**1**	**2**	—
Mountain Lion	San Diego	F	Adult	**2**	**2**	—
Mountain Lion	Modoc	M	Adult	0	1	—
Mountain Lion	Yuba	F	Adult	**2**	**2**	not tested
San Joaquin Kit Fox	Kern	M	Adult	**2**	**3**	—
San Joaquin Kit Fox	Kern	F	Adult	0	2	—
San Joaquin Kit Fox	Kern	M	Adult	2	0	—
Striped Skunk	Sonoma	M	Adult	0	1	—

Other Pesticide Surveillance

When warranted, wild small- and non-game mammals and fish were tested for additional pesticides, including organophosphates and carbamates, neonicotinoids, pyrethroids, and other compounds.

A general toxicology panel (GMCS/LCMS) was performed on one mountain lion from Mono County, one gray fox from Santa Cruz County, one Mexican free-tailed bat from Santa Clara County, and for three fish mortality cases from Trinity, San Diego, and Lake counties. Results of these tests are summarized in Table 21.

In March 2023, a colony of Mexican free-tailed bats experienced a mortality event at a property in Yuba County. The property was reported to use "mothballs" to deter bats, and the attic of the property had a strong chemical smell. Crystals and a bat carcass were recovered at the scene and tested for the compound dichlorobenzene. The crystals tested positive for 1,4-Dichlorobenzene. Liver tissue from the bat carcass tested negative, possibly due to low sample size, tissue volume, and relatively high reporting limit for tested tissue sample.

Cholinesterase levels were tested in a gray wolf from Lassen County and levels were above thresholds that indicate exposure to cholinesterase-inhibiting compounds.

Table 21. Results from GCMS/LCMS screenings conducted on non-game wildlife and fish submitted to the Wildlife Health Laboratory in 2023. No significant findings (NSF) are noted for results where the no analytes were detected, or detections were expected within normal ranges. *Fish cases were not included in small game and non-game AR surveillance summaries.

Species	County	GCMS/LCMS Detections	Concurrent AR Exposure
Mountain Lion	Mono	caffeine	Brd, brm, chl (Trace); diph (520 ppb)
Gray Fox	Santa Cruz	chlorpyrifos	brm (2400 ppb)
Mexican Free-tailed Bat	Santa Clara	NSF	—
Pooled fish fry	Trinity	NSF	—
Spotted Bay Bass*	San Diego	p,p'-DDE	Not detected
Moray Eel*	San Diego	p,p'-DDE	brd (Trace)
Bullhead Catfish	Lake	Fluridone; Endothall; cocaine; nicotine	—
Cleark Lake Hitch	Lake	NSF	—

RECENT WILDLIFE-RODENTICIDE LEGISLATION AND CURRENT RODENTICIDE-USE TRENDS

Evaluation of Assembly Bill (AB) 1788

On January 1, 2021, a temporary moratorium was placed on the public sales and use of SGARs in California (AB1788). CDFW proposed guidelines to monitor the effects of implementing AB1788, while also continuing long-term monitoring and surveillance efforts in non-target wildlife, given the long half-lives of many SGARs and their ability to bioaccumulate in the livers of animals[2].

The CDFW 2022 annual report summarized the CDFW-led short-term evaluation of the efficacy of AB1788, which entailed assessing cases of exposure in animals born or hatched after January 1, 2021 and any cases of acute toxicosis[2]. Detections of AR compounds in wildlife born or hatched after implementation of AB1788 could indicate exposure rates under the new restrictions; however, it is possible that mammals could have been exposed in utero prior to implementation of the law[5-11]. Additionally, wildlife of any age that succumbed to acute toxicosis in 2022 were likely to have been exposed to compounds recently and in concentrations high enough to cause coagulopathy and death, rather than chronic exposure accumulating over time. It is important to note, however, that most wildlife had more than one analyte detected in their livers belonging to both FGARs and SGARs. Furthermore, it is important to acknowledge that there is no minimum threshold concentration indicative of anticoagulant rodenticide toxicosis. Determining whether toxicosis was due to either an FGAR or SGAR is challenging in the presence of multiple analytes and lack of empirical data on cumulative effects. The CDFW 2022 annual report indicated that, despite the implementation of AB1788 that restricted SGAR-use, non-target wildlife was still at risk of exposure and toxicosis.

In 2023, we detected anticoagulant rodenticide exposure in 71.9% (92/128) of non-target wildlife tested. Despite the long-half lives of SGARs, which may persist in liver tissues for upwards of six to 12 months and potentially beyond (i.e., brodifacoum can have a half-life of approximately 350 days in liver tissues[12]), exposures detected in 2023 were most likely related to use after AB1788 was implemented (January 1, 2021). In birds that were tested, 26 individuals were exposed to one or more SGARs, resulting in 45 SGAR detections; 15 individual birds were exposed to one or two FGARs, resulting in 18 FGAR detections. In non-game mammals, 44 individuals were exposed to one or more SGARs, resulting in 79 SGAR detections; 44 individual non-game mammals were exposed to one or more FGARs, resulting in 60 FGAR detections. In big game mammals (black bear) tested, five individuals were exposed to SGARs, resulting in six SGAR detections; two individual black bears were exposed to FGARs, resulting in two FGAR detections. For all non-target wildlife with quantitated anticoagulant rodenticide liver concentrations, we found an average (mean ± standard error of the mean) liver concentration of 310.0 ± 65.2 ppb and 302.2 ± 61.7 ppb for SGARs and FGARs in wildlife tested in 2023, respectively (Figure 12; Figure 13; Table 22).

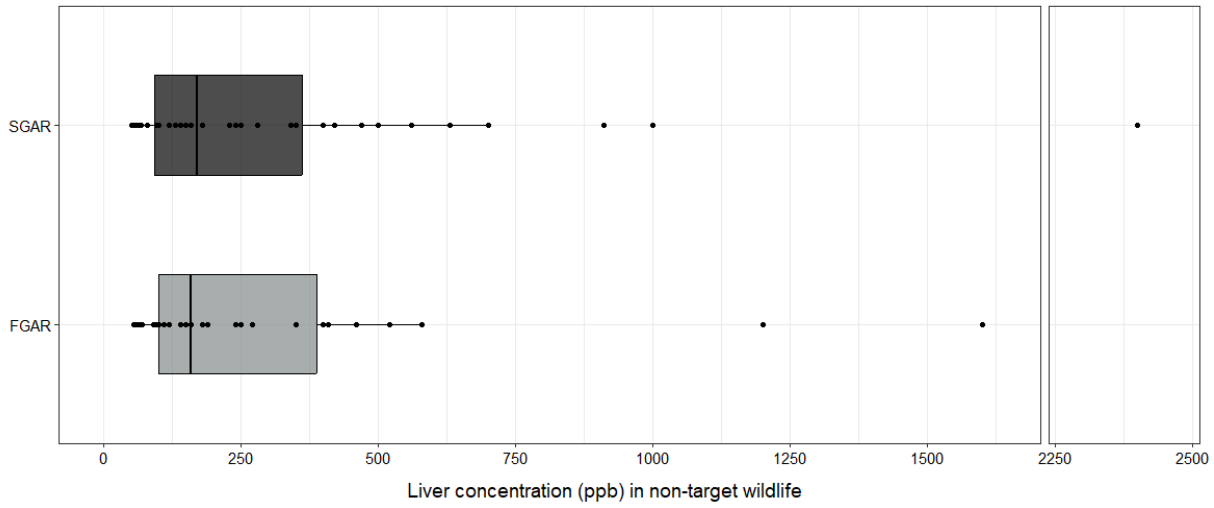

Figure 12. Box plot to visualize FGAR and SGAR concentrations (ppb) in the livers of 74 of the 128 tested wild non-game mammals submitted to the Wildlife Health Laboratory in 2023 where detectable levels were able to be quantitated. This figure does not include instances of trace level detections (see Table 22). Box plot summary can be found in Appendix 1.3.

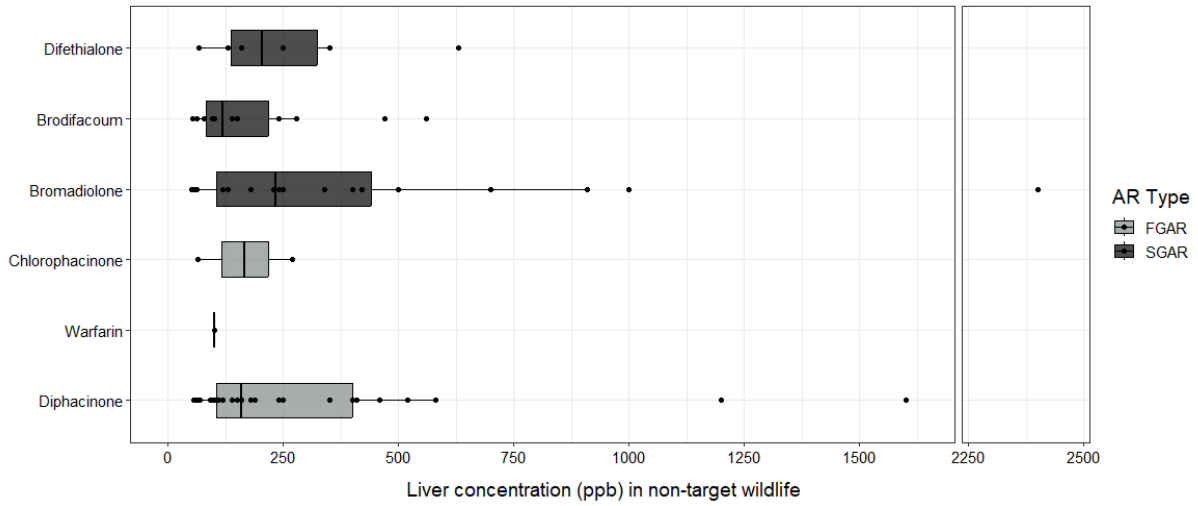

Figure 13. Box plot to visualize AR analyte concentrations (ppb) in the livers of 74 of the 128 tested all non-target avian, game, and non-game wildlife submitted to the Wildlife Health Laboratory in 2023 where detectable levels were able to be quantitated. This figure does not include instances of trace level detections (see Table 22). Box plot summary can be found in Appendix 1.4.

Table 22. AR concentrations (ppb) and number of trace detections in the livers of all non-target avian, game, and non-game wildlife submitted to the Wildlife Health Laboratory in 2023. Summary includes concentration mean and standard error (SE) of the mean, range, and number of trace detections.

AR Type	Analyte	Mean ± SE (ppb)	Range (ppb)	No. of Trace Detections
FGAR	Diphacinone	317.4 ± 66.9	56 –1600	27
	Warfarin	100	100	1
	Chlorophacinone	167.5 ± 102.5	65 – 270	19
SGAR	Bromadiolone	414.3 ± 121.8	52 – 2400	28
	Brodifacoum	180.4 ± 42.1	53 – 560	46
	Difethialone	264.7 ± 83.4	68 – 630	14

A juvenile Cooper's Hawk with rat prey in Yolo County. Photo: Ryan Bourbour, CDFW

Rodenticide surveillance and the changing rodenticide landscape

Continued rodenticide exposure in wildlife and changing patterns of state-wide use highlight the need for ongoing and adaptive surveillance efforts. Exposure to rodenticide compounds continues to be a risk to non-target wildlife in California (Table 23; Table 24). According to DPR's Pesticide Use Reporting (PUR) data, the number of rodenticide applications used for the control of commensal rodents (collectively: ARs, bromethalin, and cholecalciferol) have remained relatively constant from 2013 to 2022 (Figure 14). Notably, legislation that aims to protect non-target wildlife from anticoagulant rodenticide exposure, such as AB1788, may have implications on the types of compounds applied and rates different compounds are applied throughout the state (Figure15).

Following the implementation of AB1788 on January 1st, 2021, the PUR data shows a decline in the reported use of restricted SGARs and the increase in use of FGARs, bromethalin, and cholecalciferol (Figure 15).

Understanding the rodenticide exposure rates in non-target wildlife populations across California is challenging because in addition to long-term surveillance of tested animals, a systematic biomonitoring approach is needed. Moving forward, rodenticide surveillance efforts that reflect the evolving rodenticide landscape will be important, as different rodenticide types require different sampling and testing methods, validation methods, and data interpretation. Increases in the reported use of other rodenticides that are replacing restricted ARs create the need for adaptive surveillance strategies to inform pest management and conservation decisions. However, these adaptive efforts may be challenging to implement. For example, screening for both ARs and neurotoxic bromethalin multiplies the financial cost of pesticide screening for a single animal. Given the emerging evidence of both primary and secondary exposure risks to non-target wildlife that are tested for bromethalin[13,14] (Tables 11,12, 18–20, 24), continued surveillance and additional resources from regulating agencies are warranted to facilitate systematic monitoring of both AR and non-AR exposure for the biomonitoring of California's wildlife.

Table 23. Summary of anticoagulant rodenticide exposure and toxicosis rates from CDFW WHL Annual Reports 2020–2023[2,3,4].

Year	Total Submitted to WHL	Total Tested for ARs	Total Exposed to ARs	% Exposed to ARs	Total Confirmed Toxicosis	% Toxicosis Confirmed
2020	1,040	159	108	67.9	24	22.2
2021	1,020	250	175	70.0	19	10.9
2022	1,543	158	128	81.0	18	14.1
2023	1,250	128	92	71.9	11	12.0

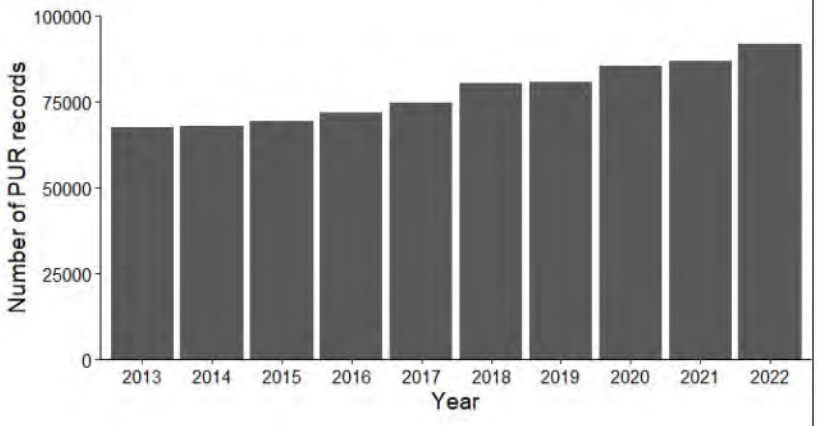

Figure 14. The total number of Pesticide Use Reporting (PUR) records for ARs, bromethalin, and cholecalciferol in California between 2013–2022. PUR data shown in graph was obtained from DPR's California Pesticide Informational Portal; 2023 PUR data was not available when this report was written. The PUR records in the figures do not indicate pounds of product or active ingredients applied.

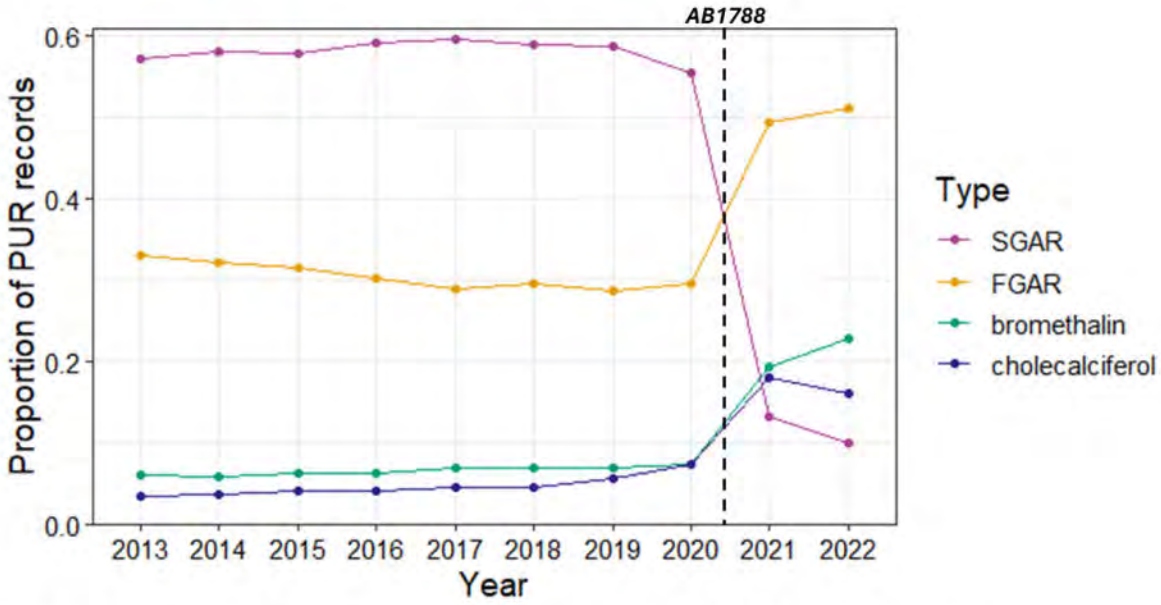

Figure 15. Proportions of the number of rodenticide applications for ARs, bromethalin, and cholecalciferol according to Pesticide Use Reporting (PUR) records in California 2013–2022. PUR data was obtained from DPR's California Pesticide Informational Portal. The black dotted line represents implementation of AB1788 on January 1st, 2021. The 2023 PUR data was not available when this report was written.

Bobcat at Semitropic Ecological Reserve. Photo: CDFW Science Institute & Lands Program

Table 24. Summary of bromethalin exposure rates for a subset of commonly tested non-target wildlife reported in CDFW WHL Annual Reports 2021–2023[2,3]. Between 2021 and 2023, CDFW detected bromethalin in 22.0% (74/338) of non-target mammals tested.

Bromethalin Detections in Non-target Wildlife (2021-2023)	
Black Bear	30.3% (10/33)
Mountain Lion	15.3% (11/72)
Bobcat	15.6% (15/96)
Coyote	20% (3/15)
Gray Fox	25% (10/40)
Raccoon	64.7% (11/17)
Striped Skunk	50% (6/12)

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An adult Great Horned Owl hunting from an artificial raptor perch in a Yolo County orchard. Photo: Ryan Bourbour, CDFW

APPENDIX 1

1.1. Summary statistics from box plot in Figure 3; calculated with *geom_boxplot* command in the R package *ggplot2*. Concentration numbers reported are in parts per billion (ppb).

Avian - Figure 3

Compound	Lower Q1	Median	Upper Q3	Min	Max	Outliers
Diphacinone	100	120	150	96	150	460
Bromadiolone	58	63	180	57	180	—
Brodifacoum	72.75	120	250	53	280	560
Difethialone	83.5	99	114.5	68	130	—

1.2. Summary statistics from box plot in Figure 11; calculated with *geom_boxplot* command in the R package *ggplot2*. Concentration numbers reported are in parts per billion (ppb).

Nongame - Figure 11

Compound	Lower Q1	Median	Upper Q3	Min	Max	Outliers
Diphacinone	110	160	400	56	580	1600, 1200
Warfarin	100	100	100	100	100	
Chlorophacinone	116.25	167.5	218.75	65	270	
Bromadiolone	180	340	600	52	1000	2400
Brodifacoum	88.5	118.5	222.5	63	222.5	470
Difethialone	205	250	300	160	350	

1.3. Summary statistics from box plot in Figure 12; calculated with *geom_boxplot* command in the R package *ggplot2*. Concentration numbers reported are in parts per billion (ppb).

All non-target - Figure 12

Compound	Lower Q1	Median	Upper Q3	Min	Max	Outliers
SGAR	100	160	387.5	56	580	1200, 1200, 1600
FGAR	92.5	170	362.5	52	700	910, 1000, 2400

1.4. Summary statistics from box plot in Figure 13; calculated with *geom_boxplot* command in the R package *ggplot2*. Concentration numbers reported are in parts per billion (ppb).

All non-target - Figure 13

Compound	Lower Q1	Median	Upper Q3	Min	Max	Outliers
Diphacinone	105	160	400	56	580	1200, 1200, 1600
Warfarin	100	100	100	100	100	
Chlorophacinone	116.25	167.5	218.75	116.25	270	
Bromadiolone	105.75	235	440	105.75	910	1000, 2400
Brodifacoum	83.5	120	217.5	83.5	280	470, 560
Difethialone	137.5	205	325	137.5	350	630

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News Releases, 2022-Present

Poison Detection in Wild Pigs Brings Attention to Pesticide Exposure in Hunter-Harvested Wildlife

July 30, 2025

Wild pigs in the Monterey County area were exposed to pesticide bait containing the anticoagulant rodenticide diphacinone, according to findings by the California Department of Fish and Wildlife's (CDFW) Wildlife Health Lab (WHL) and the California Animal Health and Food Safety Laboratory in Davis.

In March, a wildlife trapper reported multiple observations of blue muscle or fat found in wild pigs. The blue tissue can be a sign of rodenticide bait ingestion. CDFW's WHL investigated, finding the anticoagulant rodenticide diphacinone in the stomach and liver contents of one of the wild pigs that was recovered with blue tissues.

Wildlife can be inadvertently exposed to rodenticides either by eating rodenticide bait or by eating other animals that have ingested rodenticides. Rodenticide baits often contain dye to identify them as a poison. Blue-colored muscle or fat may be a sign that game meat has been contaminated by rodenticides, although this blue discoloration may not always be present. CDFW urges hunters to always use caution when harvesting game animals and be aware of potential risks.

“Hunters should be aware that the meat of game animals, such as wild pig, deer, bear and geese, might be contaminated if that game animal has been exposed to rodenticides,” said CDFW Pesticide Investigations Coordinator Dr. Ryan Bourbour. “Rodenticide exposure can be a concern for non-target wildlife in areas where applications occur in close proximity to wildlife habitat.”

A 2018 study of anticoagulant rodenticide exposure in game animals across California found anticoagulant rodenticide residue in 10 out of 120 (8.3%) of the wild pig and 10 out of 12 (83%) of the bear tissue samples collected largely from animals that were frequenting agricultural or residential areas where rodenticides are commonly/more likely to be utilized.

CDFW encourages hunters to report unusual findings in harvested wildlife, including blue tissue, and not to consume any part of an animal with blue fat or muscle or other abnormalities. Incidents may be reported to the CDFW’s Wildlife Health Lab at WHLab@wildlife.ca.gov or (916) 358-2790.

Pesticide applicators are urged to take measures when applying rodenticides so as not to expose wildlife. Prior to application, it is important to ensure non-target wildlife are not using the area where the pesticide is to be applied. It is also important to use appropriate bait stations and application methods that exclude access to non-target species. Using an integrated pest management approach for rodent control may help reduce the opportunities for rodenticide exposure for non-target wildlife.

For questions about pesticide use and regulations, or to report misuse, contact your local county agricultural commissioner’s office. For Monterey County, call (831) 759-7325.

Visit CDFW’s human-wildlife conflicts web page for more information and resources for managing squirrels and other rodents.

###

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Categories: Environment, Hunting, Wildlife, Wildlife Health

Tagged: Wild Pig | Pesticide Esposure | Rodenticides | Wildlife Health Lab

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Department of Pesticide Regulation
Pesticide Registration Branch
November 16, 2018

An Investigation of Anticoagulant Rodenticide Data Submitted to the Department of Pesticide Regulation

Introduction

In 1999, the California Department of Pesticide Regulation (DPR) placed pesticide products containing brodifacoum into reevaluation in response to a request from the California Department of Fish and Game (now the California Department of Fish and Wildlife [DFW]). In 2013, DPR assessed available data on second-generation anticoagulant rodenticides (SGARs) currently registered in California (brodifacoum, bromadiolone, difenacoum, and difethialone) and determined that the use of SGARs presented unmitigated risks related to persistent residues in target animals, resulting in impacts to non-target wildlife.

To mitigate the risks identified by the assessment, effective July 1, 2014, DPR designated the SGAR active ingredients brodifacoum, bromadiolone, difenacoum, and difethialone as California restricted materials. As a result, rodenticides containing the four active ingredients can only be sold by licensed dealers and purchased by certified applicators (DPR, 2014). DPR also added additional use restrictions and revised the definition of a private applicator. Products containing first-generation anticoagulant rodenticides (FGARs), which include warfarin, chlorophacinone, and diphacinone, were not included in these regulatory changes.

Since implementation of the regulatory change in 2014, DPR continued to receive and analyze data regarding exposure to non-target wildlife from anticoagulant rodenticides (ARs). Thorough analysis is required to fully assess the impact of these regulatory changes over time and aid in determining if further regulatory action is warranted. This report incorporates information and data from a variety of sources, including peer-reviewed scientific publications, statewide sales and use reporting data, and unpublished wildlife incident and mortality data. Publications and data utilized in the decision-making process are reviewed and discussed below.

On December 22, 2017, DPR received a letter, accompanied by data and exhibits, from the law offices of Michael W. Graf, on behalf of Raptors Are the Solution and Project Coyote, requesting that the following seven pesticide active ingredients be placed into reevaluation based on significant impacts on wildlife health and the environment: 1) brodifacoum, 2) bromadiolone, 3) difethialone, 4) difenacoum, 5) diphacinone, 6) chlorophacinone, and 7) warfarin. DPR currently registers rodenticides containing these active ingredients for sale and use in California.

This report analyzes the data and exhibits submitted to DPR by Mr. Graf, as well as all information and data that has been submitted to DPR by DFW (2014-2018). It also incorporates information and data from a variety of sources, including statewide sales and use reporting data, and unpublished wildlife incident and mortality data.

Background

Anticoagulant rodenticides are typically classified as either first-generation or second-generation. First-generation anticoagulants, such as warfarin, though initially efficacious, began to lose their effectiveness. The appearance of rats and mice resistant to warfarin necessitated the development of alternatives. This eventually led to the development of SGARs, brodifacoum, bromadiolone, difethialone, and difenacoum. FGARs and SGARs share a similar mechanism of action, but SGARS have increased toxicity, prolonged half-lives, and increased lipophilicity.

The increased toxicity of the SGARs corresponds to lower effective doses. For instance, in rats, warfarin has an oral LD_{50} of 58.0 mg/kg, whereas brodifacoum has an oral LD_{50} of 0.26 mg/kg (U.S. EPA, 2004; Redfern et al., 1976; Thomson, 1988). Accordingly, it may take multiple feedings of a FGAR to reach a lethal dose, but a single feeding of a SGAR can result in lethality. Table 1 presents a comparison of the most sensitive LD_{50} values for birds and mammals (not just rats) for the ARs.

Toxicity is one component of the ARs' efficacy in animals. Due to their mechanism of action, there is a delay between consumption of a lethal dose and death of the exposed organism. As a result, the target organism may continue to consume the bait. In the case of an SGAR, this allows for super-lethal concentrations of the rodenticide to accumulate in its body. Secondary non-target wildlife exposure may occur, when non-target wildlife feed on the exposed target pest.

The SGARs are more persistent than FGARs in the livers of animals that have been exposed. For example, warfarin has a hepatic (liver) half-life of 26.2 days, whereas brodifacoum has a hepatic half-life of up to 350 days (Table 2; U.S. EPA, 2004). The significantly extended hepatic half-lives for SGARs means that an animal that ingested the anticoagulant can potentially carry that compound for years, as compared to days or months for an FGAR.

Finally, the increased lipophilicity of the SGARs can increase the amount of AR that is absorbed to the tissues. For example, brodifacoum has an octanol-water partition coefficient (K_{ow}) that is approximately five orders of magnitude higher than warfarin (Table 3). This suggests that if two animals are dosed with equal amounts of brodifacoum and warfarin, the animal dosed with brodifacoum will have a higher initial concentration in its liver because brodifacoum is more lipophilic. A higher initial concentration in the liver tissue means that there will be detectable residues in the liver for a longer time, even if the rate of decline is the same for both compounds. This, in effect, further amplifies the persistence of the SGARs.

Table 1 – Comparison of toxicity values for birds and mammals for ten rodenticides.

Type of Rodenticide	Active Ingredient	Most Sensitive LD_{50} for Birds (mg ai/kg bw) [a, b]	Most Sensitive LD_{50} for Mammals (mg ai/kg bw) [a, b]
SGARs	**Brodifacoum**	**0.26**	**0.13**
	Bromadiolone	138	0.56
	Difenacoum	66	0.45
	Difethialone	**0.26**	**0.29**
FGARs	Chlorophacinone	>100	0.49
	Diphacinone	96.8	0.2
	Warfarin	620	2.5

Bold font represents those active ingredients that have similar LD_{50} values for mammals and birds. The other active ingredients have a substantial difference between the LD_{50} values for mammals and birds.

[a] Data summarized from DPR, 2013

[b] LD_{50} values presented in units of milligrams of active ingredient per kilogram of body weight

Table 2 – Hepatic half-lives of seven ARs in the livers of target species.

Type of Rodenticide	Active Ingredient	Hepatic half-lives (Days) [a]
SGARs	Brodifacoum	113.5-350
	Bromadiolone	170-318
	Difenacoum	118
	Difethialone	126
FGARs	Chlorophacinone	< 2
	Diphacinone	3
	Warfarin	26.2

[a] Data summarized from DPR, 2013

Table 3 – Octanol-water partition coefficient (K_{ow}) values for seven ARs.

Type of Rodenticide	Active Ingredient	Log K_{ow}
SGARs	Brodifacoum	8.5 [a]
	Bromadiolone	4.3 [b]
	Difenacoum	7.6 [c]
	Difethialone	9.82 [d]
FGARs	Chlorophacinone	1.98 [e]
	Diphacinone	4.3 [f]
	Warfarin	2.70 [g]

References: [a] U.S. EPA, 2016-a; [b] U.S. EPA, 2016-b; [c] U.S. EPA, 2007; [d] U.S. EPA, 2016-c; [e] U.S. EPA, 2015-a; [f] U.S. EPA, 2012; [g] U.S. EPA, 2015-b

Descriptions of Data and Exhibits Submitted to DPR by Michael Graf

- **California Department of Fish and Wildlife (DFW) AR Exposure Cases**

The Department of Fish and Wildlife receives animals from various sources including wildlife rehabilitation centers and County Agricultural Commissioners. These animals are generally necropsied by DFW and then liver samples are sent to the California Animal Health and Food Safety Laboratory at UC Davis for AR testing. DFW then submits loss reports (i.e., necropsy reports) to DPR for non-target wildlife that test positive for exposure to rodenticides. DPR examines the submitted loss reports, compiles them in a database, and analyzes the data (Table 4, Figures 1-5).

There are several limitations in the loss reports provided to DPR that preclude the analysis of trends or overall exposure. First, DFW only provides reports for non-target wildlife that test positive for exposure to rodenticides. DFW does not inform DPR of the total number of animals tested. Second, the animals are not collected randomly. For a sample to be representative of a population, the data must be collected randomly (Ott and Longnecker, 2010). For example, when distressed animals are brought to wildlife rehabilitation centers, they are not collected randomly, are not healthy animals and are, therefore, not representative of the general population of healthy animals. Third, when wildlife rehabilitators suspect that an animal may have been exposed to rodenticides, they send the body to DFW for necropsy. This further biases the data collected toward positive tests for rodenticide exposure. Finally, DFW prioritizes which animals to necropsy and/or test for rodenticide exposure, and the criteria that DFW uses to prioritize animals for necropsy is unknown. This means the data may potentially have multiple levels of bias which result in a high percent of animals testing positive for AR exposure. This does not mean that the data is invalid, or that the data does not have value from a regulatory perspective. However, it must be noted that the data is not representative of the general population of all wild animals, conclusions drawn from these data have to explain the caveats and uncertainties including its limitations in representing the percentage of all wild animals that may be exposed to anticoagulant rodenticides. DPR has requested more information on DFW's methodology and selection procedures.

Table 4 – DPR analysis of AR exposure rates based on DFW loss reports

Parameter	2014	2015	2016	2017	2018
Total Reported Animals Tested	18	42	56	24	12
No. of Reported Mammals Tested	16	28	45	14	6
No. of Reported Birds Tested	2	14	10	10	6
No. of Reported Non-Bird/Mammals Tested	0	0	1	0	0
No. of Reported Animals with Detectable Levels of ARs	16 / 18	41 / 42	52 / 56	20 / 24	12 / 12
Maximum No. of ARs Detected	5	4	5	5	4
Minimum No. of ARs Detected	0	0	0	0	1
Mean No. of ARs Detected	2.5	2.1	2.2	2.5	2.4
No. of Reported Animals with Detectable Levels of FGARs	9 / 18	21 / 42	16 / 56	9 / 24	3 / 12
No. of Reported Animals with Detectable Levels of Chlorophacinone	1 / 18	3 / 42	3 / 56	6 / 24	0 / 12
No. of Reported Animals with Detectable Levels of Diphacinone	9 / 18	18 / 42	15 / 56	6 / 24	3 / 12
No. of Reported Animals with Detectable Levels of Warfarin	1 / 18	1 / 42	1 / 56	1 / 24	0 / 12
No. of Reported Animals with Detectable Levels of SGARs	16 / 18	35 / 42	51 / 56	19 / 24	12 / 12
No. of Reported Animals with Detectable Levels of Brodifacoum	14 / 18	32 / 42	48 / 56	19 / 24	11 / 12
No. of Reported Animals with Detectable Levels of Bromodiolone	14 / 18	18 / 42	32 / 56	13 / 24	7 / 12
No. of Reported Animals with Detectable Levels of Difenacoum	1 / 18	2 / 42	0 / 56	3 / 24	1 / 12
No. of Reported Animals with Detectable Levels of Difethialone	5 / 18	15 / 42	23 / 56	12 / 24	7 / 12

Notes:

This table includes all data provided to DPR by DFW from 2014 to 2018.

ARs: Anticoagulant Rodenticides

FGARs: First Generation Anticoagulant Rodenticides

SGARs: Second Generation Anticoagulant Rodenticides

Figure 1 – DPR's preliminary analysis of SGAR non-target wildlife exposure rates based on loss reports submitted by DFW.

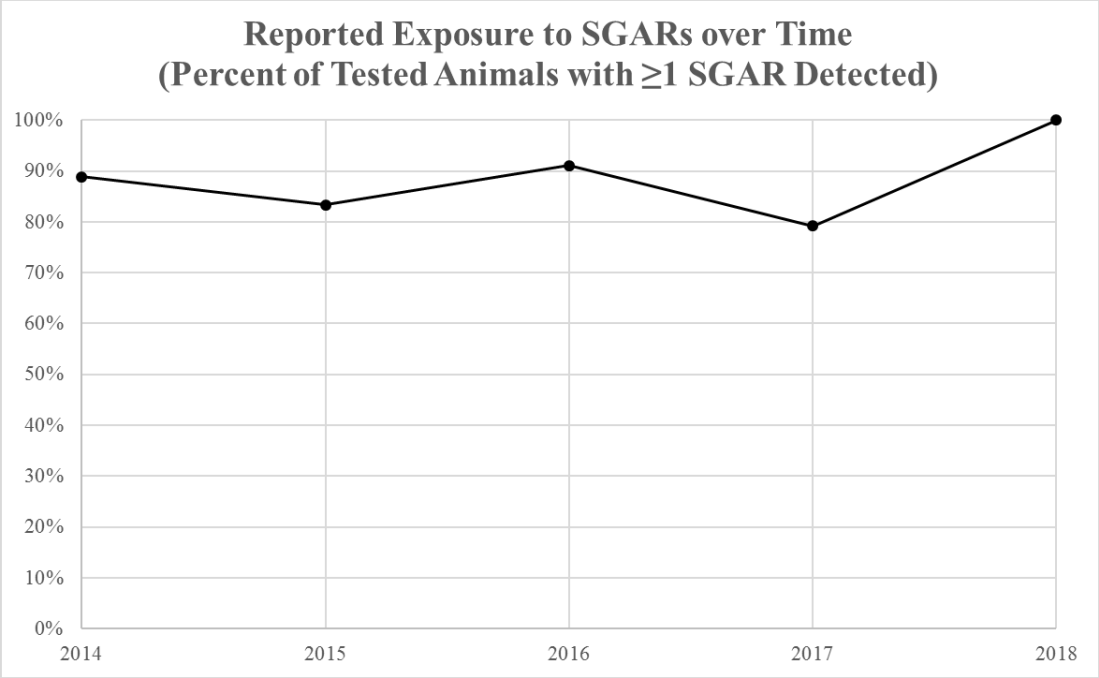


Figure 2 – Exposure rates of individual SGAR active ingredients from 2014-2018 (chart created by DPR scientists from non-target wildlife loss reports submitted by DFW).

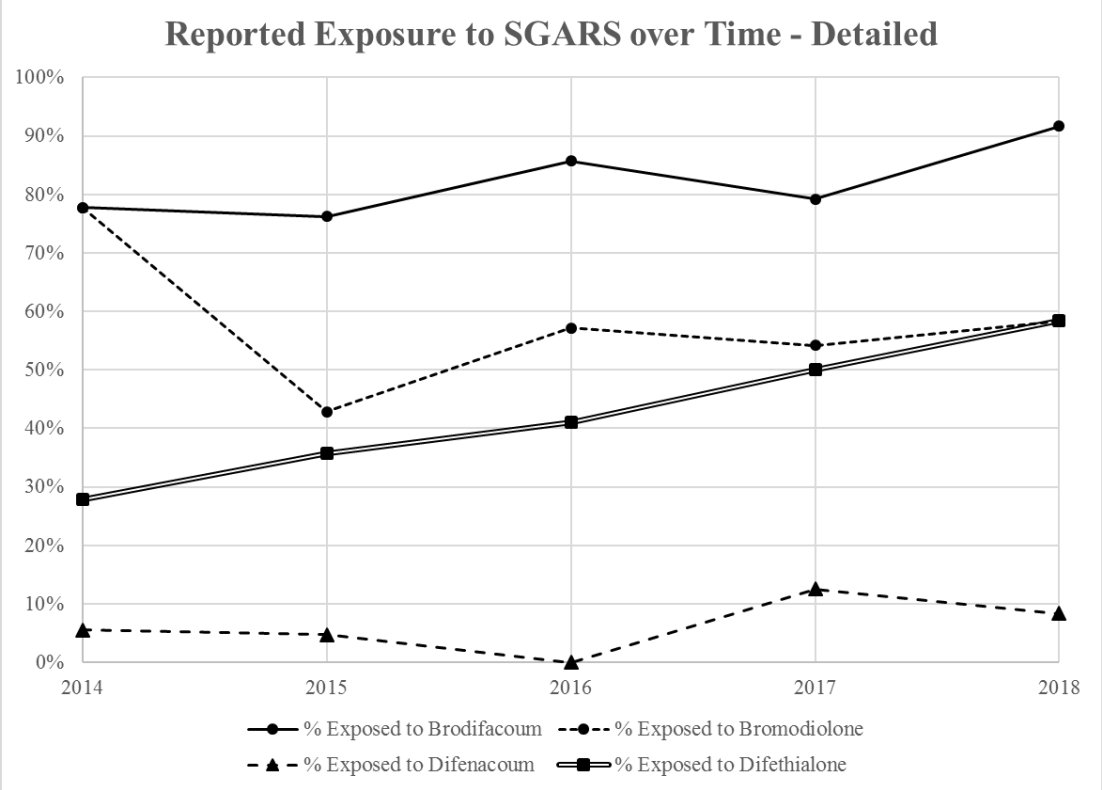


Figure 3 – DPR's preliminary analysis of FGAR non-target wildlife exposure rates based on loss reports submitted by DFW.

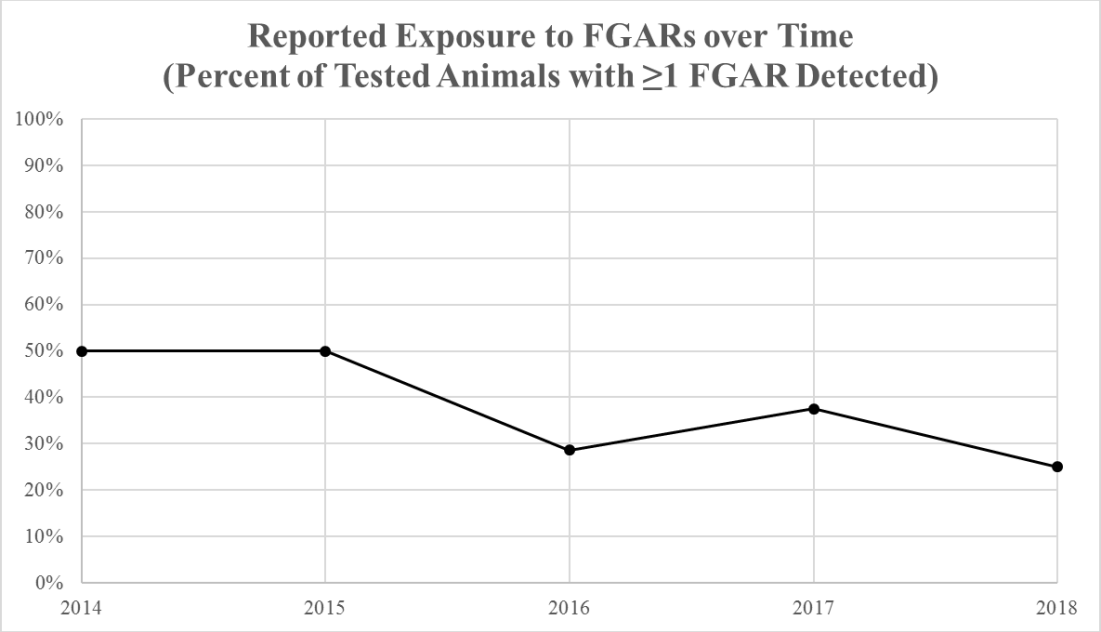


Figure 4 – Exposure rates of individual FGAR active ingredients from 2014-2018 (chart created by DPR scientists from non-target wildlife loss reports submitted by DFW).

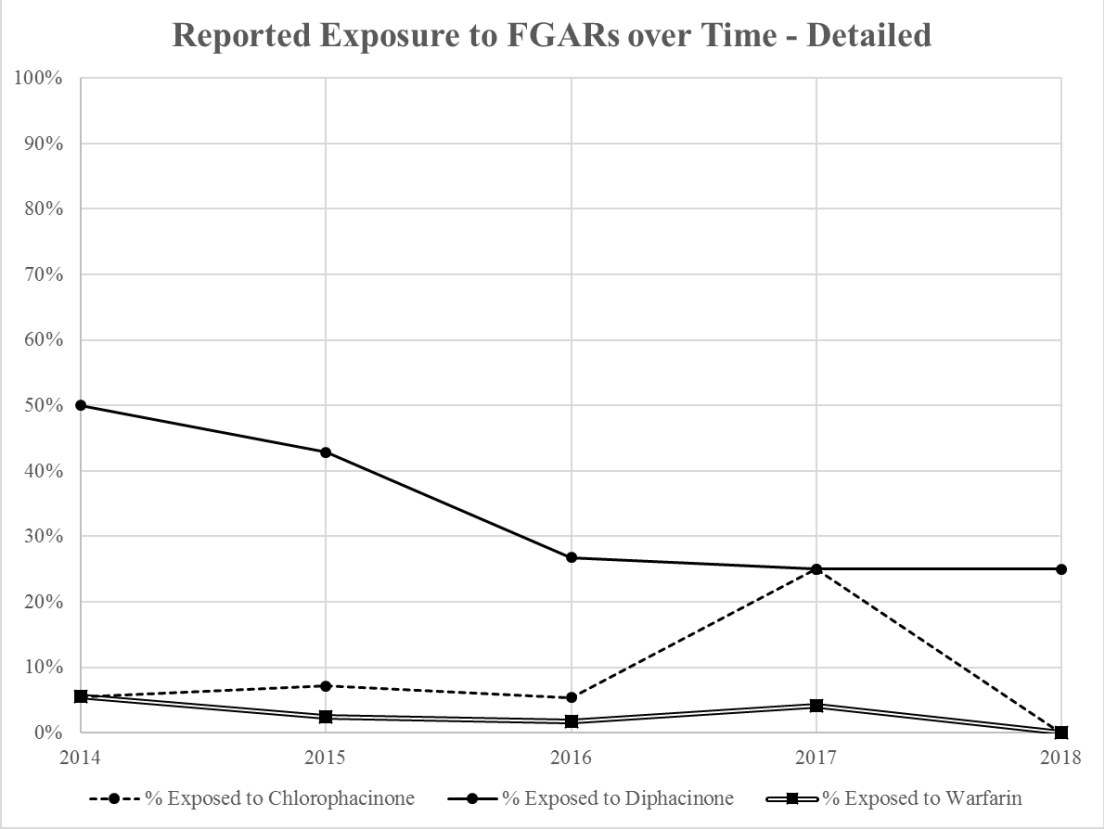
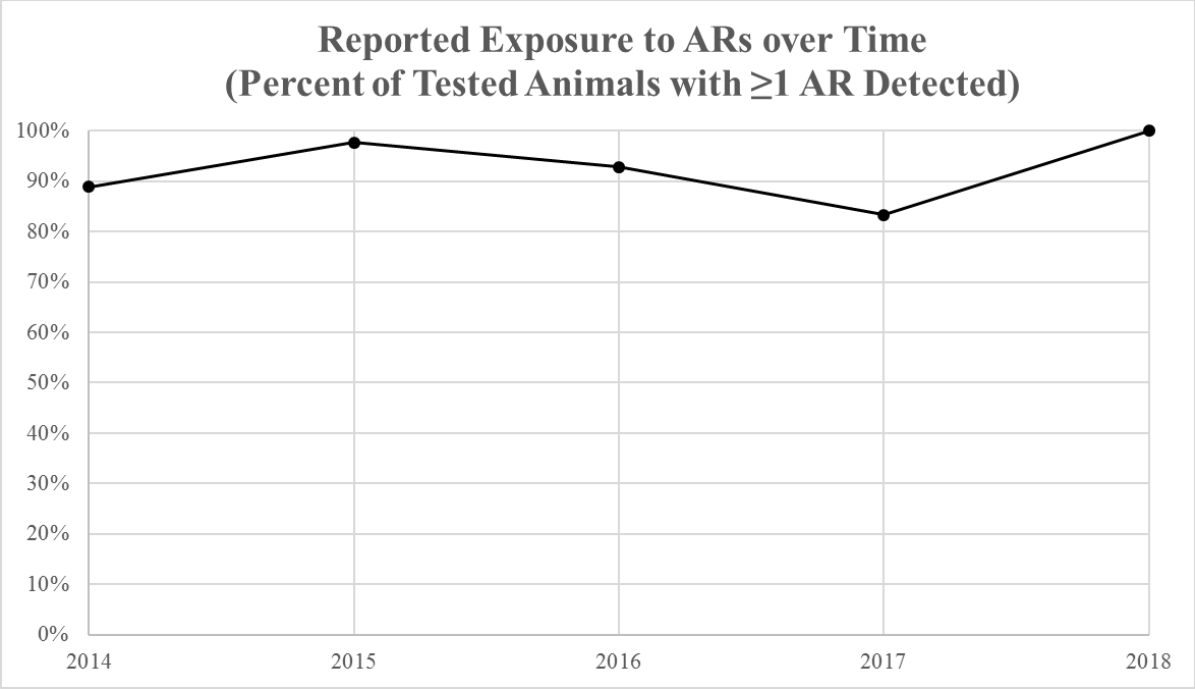


Figure 5 – DPR's preliminary analysis of AR (all ARs, 1st and 2nd generation) exposure rates based on non-target wildlife loss reports submitted by DFW.



- **DFW Mountain Lion Database**

DFW and Michael Graf both independently provided DPR with the same database of mountain lion AR exposure data. DFW did not provide DPR with a written account of how this data was collected, but in a recent (October 4, 2018) meeting between DFW and DPR scientists, DFW scientists stated that the rodenticide screening for mountain lions was part of a two-year grant in which DFW tested every mountain lion available. DFW stated that many of these mountain lions were killed through depredation permits, but some were also killed in vehicular collisions, as well as other causes of death. Therefore, although the sample collection was not completely random, there is minimal selection bias. DPR scientists conducted an independent analysis of this data. At this time, DPR has excluded four mountain lions without a date of death from its analysis. If additional information is provided by DFW, DPR will include all mountain lions in its analysis.

The exposure rates found in these mountain lions are high. However, given the long hepatic half-lives of the SGARs, it is possible that the mountain lions were exposed before the regulations went into effect (July 1, 2014). Difenacoum has the shortest hepatic half-life (118 days) of the SGARs. A half-life is the time required for a concentration to decrease by half in a given media (e.g., the liver). This should not be confused with the amount of time it takes for a chemical to degrade, or to be eliminated from an animal's body completely. As a rule, the length of time needed for a chemical to degrade (or metabolize) to less than one-percent of the initial concentration (i.e., 99% removal) is seven half-lives. Although this data cannot be used to evaluate the efficacy of the 2014 regulations, it can be used to compare exposure rates among different rodenticide compounds. Among mountain lions that were tested, the AR with the highest exposure rate is brodifacoum, followed by bromadiolone (Table 5, Figures 6 and 7).

Table 5 – DPR's independent analysis of the DFW Mountain Lion Database (excluding four animals without a date of death).

Parameter	2015-2016
Total Number of Animals Reported	64
Percent of Reported Animals with Detectable Levels of ARs	92%
Maximum Number of ARs Detected	6
Minimum Number of ARs Detected	0
Mean Number of ARs Detected	2.7
Percent of Reported Animals Exposed to Detected FGARs	67%
Percent of Reported Animals Exposed to Chlorophacinone	11%
Percent of Reported Animals Exposed to Diphacinone	59%
Percent of Reported Animals Exposed to Warfarin	8%
Percent of Reported Animals Exposed to Coumatetralyl	0%
Percent of Reported Animals Exposed to Detected SGARs	92%
Percent of Reported Animals Exposed to Brodifacoum	91%
Percent of Reported Animals Exposed to Bromodiolone	72%
Percent of Reported Animals Exposed to Difenacoum	0%
Percent of Reported Animals Exposed to Difethialone	25%
Notes:	
This table includes all data provided to DPR by DFW from 2014 to 2018.	
AR: Anticoagulant Rodenticide	
FGAR: First Generation Anticoagulant Rodenticide	
SGAR: Second Generation Anticoagulant Rodenticide	

Figure 6 – Second-generation anticoagulant rodenticide (SGAR) exposure rates among tested mountain lions (bar graph created by DPR scientists using DFW data).

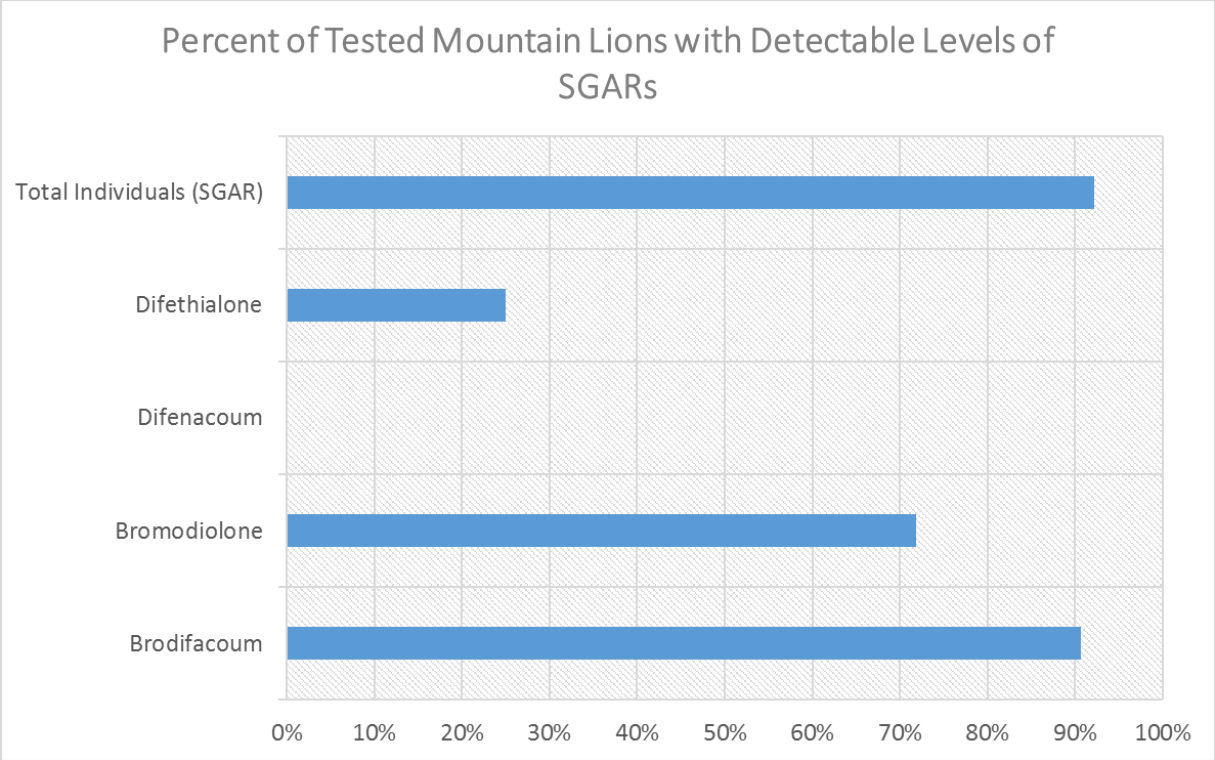
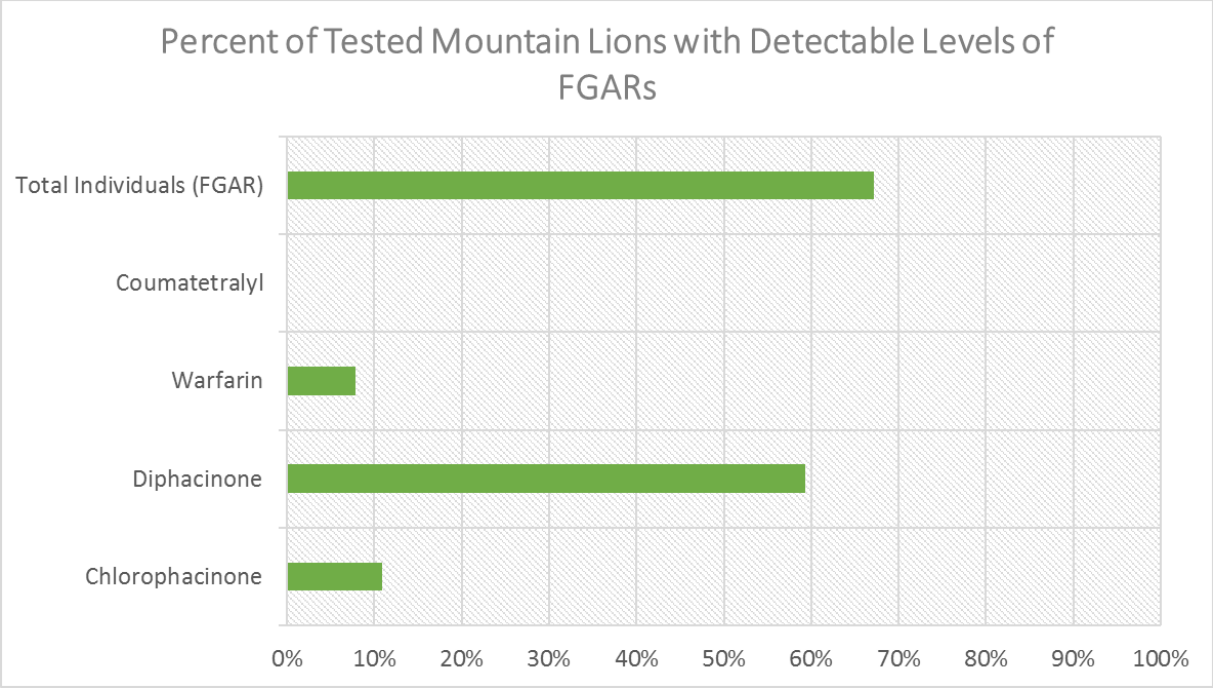


Figure 7 – First-generation anticoagulant rodenticide (FGAR) exposure rates among tested mountain lions (bar graph created by DPR scientists using DFW data).



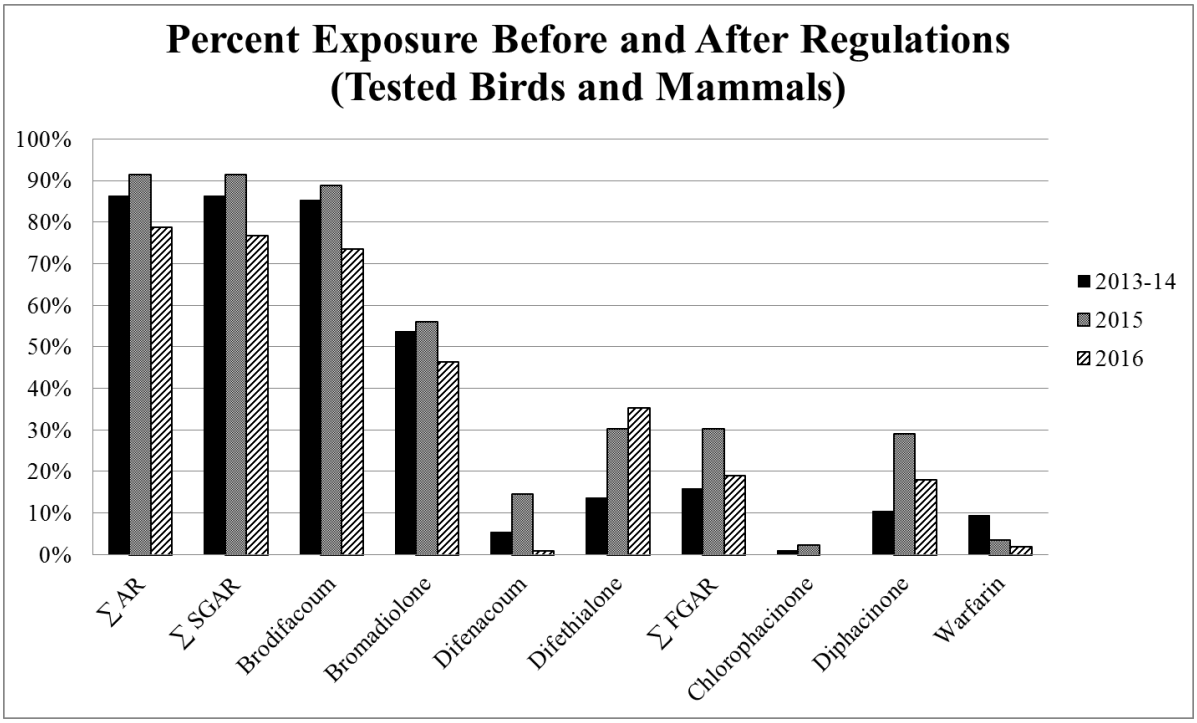
- **WildCare Wildlife Rehabilitation Center Data**

WildCare is a non-profit organization that operates a wildlife rehabilitation hospital in the San Francisco Bay Area. In 2013, DPR entered into a contract with WildCare to provide AR exposure data on non-target wildlife. In 2014, DPR renewed the contract for two more years. As of December, 2016, which is when the contract ended, WildCare provided DPR with exposure data for 115 domestic pets and 276 wild animals. Of the 115 domestic pets tested, two tested positive for exposure to FGARs. Two dogs were exposed to trace amounts of diphacinone. These were the only two exposure cases among tested domestic pets.

It is important to note that the wild animals tested were not selected randomly. This dataset is biased towards distressed animals that were brought to the WildCare wildlife hospital for rehabilitation and subsequently died or were euthanized. This does not mean that this data is not valid, or that it does not have value from a regulatory perspective, but it must be noted that the data from this study is not representative of the general population of all wild animals, so it cannot be extrapolated to draw conclusions about the percent of all wild animals that are exposed to ARs.

Of the 276 wild animals tested, exposure rates were high, both before and after the new regulations took effect (Figure 8). Nearly all SGAR exposed animals were exposed to brodifacoum and many animals were exposed to more than one anticoagulant rodenticide. However, the contract ended in 2016, which was only two years after the regulations went into effect, and it is likely too soon to expect the changes in use patterns enacted with the new regulations to influence SGAR exposure rates because of their prolonged half-lives. For example, the highest recorded concentration of brodifacoum in the liver of any non-target wildlife was 2.1 ppm in a skunk. Using a half-life of 350 days, the concentration in this particular skunk's liver after one year would be approximately 1 ppm, after two years 0.5 ppm, after three years 0.25 ppm, after four years 0.125 ppm, after five years 0.0625 ppm. The minimum reporting limit for this analysis was 0.05 ppm. This means that, had this skunk not died of a bacterial infection, it could have been brought into the WildCare Wildlife Hospital five years later, and still would have had detectable (i.e., >0.05 ppm) residues of brodifacoum in its liver. However, most animals tested (n = 276) had liver concentrations much lower than 2.1 ppm.

Figure 8 – Summary of WildCare data on file with DPR. This graph was created by DPR scientists in March 2017, using raw data received from WildCare. ∑ AR, ∑ SGAR, and ∑ FGAR represent the sum of all animals that were exposed to any AR (FGAR and/or SGAR), SGAR, and FGAR, respectively.



The following eight publicly available peer-reviewed publications were submitted by Mr. Graf. DPR scientists were already aware of many of the studies. The quality of these publications varies, but all were analyzed by DPR.

- **Vyas, N.B., Kuncir, F., and C.C. Clinton, 2017, Influence of Poisoned Prey on Foraging Behavior of Ferruginous Hawks, The American Midland Naturalist, 177(1), pp. 75-83.**

The study authors conducted an observational study at two black-tailed prairie dog (*Cynomys ludovicianus*) sub-colonies that were treated with Rozol Prairie Dog Bait (0.005% chlorophacinone, a FGAR applied at a rate of 6.9 kg of formulated end-product per hectare) and one untreated black-tailed prairie dog sub-colony. The purpose of the study was to observe the foraging behavior of ferruginous hawks (*Buteo regalis*) to see if they showed a preference for foraging in the treated or the untreated sub-colonies. The two treated sub-colonies comprised a combined 16.3 hectares with 1,986 active prairie dog burrows whereas the untreated sub-colony comprised 16.8 hectares with 2,032 active prairie dog burrows. The two treated sub-colonies were separated by a dirt county road whereas the single untreated sub-colony was approximately 100 meters (m) south on the other side of a ridge with dense vegetation. The three colonies were monitored by three people (one for each colony) concurrently on Days 8, 9, 10, 11, 16, and 17 post-application. Observers were rotated daily to avoid individual bias. The parameters examined were hawk presence, duration of activity, predation, and the overall number of prairie dogs above ground.

Over the six days of observations, hawks spent a total of 708 and 203 minutes in the treated sub-colonies and untreated sub-colonies, respectively. Hawks were observed in the treated sub-colonies on each of the six days when observations were conducted, but only on four days in the untreated sub-colony. Four predations were observed in the treated sub-colonies and zero predations were observed in the untreated sub-colony. There was a significant decline in the overall number of above ground prairie dogs in the treated sub-colony, but not in the untreated sub-colony. The study authors concluded that the hawks showed a preference for foraging in the treated sub-colonies because the poisoned prairie dogs were easier to capture due to lethargy and decreased awareness. However, they also stated that "prey accessibility is affected by vegetation cover and perch availability" and that the two sub-colonies that had been treated with Rozol had more structures that hawks could use as perches (ten utility poles and 2,519 m of barbed wire fencing in the treated sub-colonies vs. no utility poles and 597 m of fencing in the untreated sub-colonies). Although this may seem like a major confounding factor, the study authors stated that the difference in the availability of structures available for hawks to use as perches did not impact the overall results because the hawks that captured prey in the treated sub-colonies were observed doing so from soaring flights, not from perches. Overall, hawks were only observed preying on prairie dogs in the treated sub-colonies, despite the fact that in the three sub-colonies the untreated sub-colony has four times more above ground prairie dogs than the treated sub-colonies. Although the sample size was small and the duration was short (a total of 19 visits by hawks and six days of observations), DPR scientists have concluded that this study is scientifically sound and provides a qualitative line of evidence that ferruginous hawks show a preference for foraging on prairie dogs that have been treated with chlorophacinone.

- **Gabriel, M.W., Woods, L.W., Wengert, G.M., Stephenson, N., Higley, J.M., Thompson, C., Matthews, S.M., Sweitzer, R.A., Purcell, K., Barrett, R.H., Keller, S.M., Gaffney, P., Jones, M., Poppenga, R., Foley, J.E., Brown, R.N., Clifford, R.L, and B.N. Sacks, 2015, Patterns of Natural and Human-Caused Mortality Factors of a Rare Forest Carnivore, the Fisher (*Pekania pennanti)* in California. PLoS ONE 10(11): e0140640.**

In this study, the study authors used histology, toxicology, and gross necropsy to determine the cause of death for 167 individual fishers (*Pekania pennant*) collected between 2007 and 2014 from two sub-populations in California. Both of these sub-populations are considered to be evolutionarily significant units by DFW (2015). The first sub-population was located in the Northern Coast and Southern Cascade mountain ranges and the second sub-population was located in the Southern Sierra Nevada. The second sub-population is listed as threatened under the California Endangered Species Act and is believed to be comprised of roughly 300-350 fishers with fewer than 120 breeding females. Fifty-two of the fishers included in this study were from the first sub-population and 115 from the second. Of the 167 fishers included in this study, 44% were males, 56% were female. In terms of age groups: 63% were adults, 19% were sub-adults, 16% were juveniles, and 2% were kits.

Overall, the cause of death was determined for 129 fishers: 70% were determined to have died from predation, 16% from natural diseases, 10% from poisoning, 2% from getting hit by cars, and 2% from other human causes. Of the 101 fishers that had their livers tested for anticoagulant exposure, 86 individuals were determined to have been exposed to one or more ARs. Animals

can be exposed to ARs without being killed by them. The criteria for diagnosing AR toxicosis as the cause of death generally requires coagulopathy without any other signs of trauma in addition to the detection of ARs in the liver. The study authors determined that AR exposure was the cause of death for 11 fishers. They stated that these 11 fishers exhibited coagulopathy and significant hemorrhage in addition to detection of ARs in the liver. It is unclear if the 11 fishers determined to have died from AR exposure had any other signs of trauma. All of the fishers that were determined to have died from anticoagulant intoxication had illegal cannabis cultivation sites in their home ranges. The mean (± SD) number of AR compounds found in the livers of dead fishers was 1.73 ± 0.91 and some fishers were found to have been exposed to as many as five different ARs. The study authors stated that cholecalciferol "was assumed to be the contributing cause of death in one male fisher from Northern California", but that fisher was also exposed to five different ARs. Another fisher was noted as displaying neurological signs and was found near an illegal cannabis cultivation site where bromethalin was also found, but bromethalin was not detected in the stomach contents, liver, urine, or kidney. However, DPR scientists recognize that bromethalin is normally detected in adipose or brain tissue, which the study authors did not test, so it is unclear if that fisher had been exposed to bromethalin. Overall, the study authors concluded that on an annual basis from 2007 to 2014, an average of 1.86 fisher toxicosis cases were noted in California. The study authors also concluded that when the first phase of the study (with 46 of 58 fishers tested from 2007-2011 exposed) was compared to the second phase of the study (with 86 of 101 fishers tested from 2012-2014 exposed) exposure to ARs increased by 6%. It is important to note that the study authors attributed the exposure of fishers to various rodenticide compounds to be associated with illegal cannabis cultivation sites, so it is likely that most of this exposure resulted from the illegal use of rodenticides (i.e., uses not in compliance with the label). Currently, most of these sites are not remediated after being discovered and dismantled. The study authors recommend that toxicants left at illegal cannabis grow sites be removed when they are shut down. This study shows that 85% of fishers that were tested for ARs are exposed, even though they are in remote forested areas, far from urban development. Considering that DPR's regulations making SGARs restricted materials went into effect in July of 2014, this study does not provide any information on the efficacy of those regulations in reducing non-target wildlife exposure rates. The restricted material designation means that these rodenticides can only be sold in California to licensed applicators, which makes it more difficult for persons engaged in illegal cannabis cultivation operations to purchasing SGARs in California, which in turn, should reduce exposure rates among these rare forest carnivores.

- **Poessel, S.A., S.W. Breck, K.A. Fox, and E.M. Gese, 2015, Anticoagulant Rodenticide Exposure and Toxicosis in Coyotes in the Denver Metropolitan Area, Journal of Wildlife Diseases, Vol. 51, No. 1, pp. 265-268.**

In this study the livers of five coyotes (*Canis latrans*) were tested for ARs. Initially, 32 coyotes were captured and fitted with radio collars to track their movements. Thirteen of the 32 collared coyotes died during the study and the study authors decided to test the livers of five coyotes (of those coyotes that died during the study) because those coyotes were noted with sarcoptic mange. This selection procedure introduced bias into the study because they only tested the livers of coyotes that they suspected had been exposed to ARs. The coyotes' liver tissue was tested for brodifacoum, bromadiolone, difenacoum, difethialone, chlorophacinone, diphacinone,

and warfarin. Additionally, one of the five coyotes tested was not collared. That coyote was euthanized because it sustained self-inflicted injuries related to being trapped. When this coyote was tested for ARs, it was noted as having 95 ppb of brodifacoum in its liver. Overall, only 36% (5 of 14) of the coyotes that died during the study were tested. All five of the coyotes whose livers were tested were determined to have been exposed to brodifacoum and one of those was noted as having been exposed to brodifacoum and bromadiolone.

There are many issues which impact this study and make some of the authors' conclusions questionable. The study authors concluded that ARs were contributing factors in at least two of the five coyotes that had their livers tested for exposure. The descriptions of these two coyotes contained some confusing statements:

> "The first case was a juvenile male (24M) found dead in open space, with no obvious external injuries or other signs of trauma. Upon necropsy, we found free blood in the abdominal cavity. A puncture wound was present on the left side of the body overlying the spleen but not penetrating the abdominal wall. The spleen was fractured and surrounded by clotted blood. We found no radiographic evidence of gunshot and no evidence of bite wounds. The interpretation for cause of death was acute severe hemorrhage, disproportionate to the amount of trauma observed. This coyote's liver was positive for brodifacoum (176 ppb)."

In the first sentence of this description the study authors state that this coyote had "no obvious external injuries or other signs of trauma" but then, two sentences later they state that a "puncture wound was present on the left side of the body." It is unclear if the study authors consider a puncture wound to be an external injury. Additionally, it does not appear that this coyote, or any of the coyotes in this study, were tested for bacterial or viral infections. The description of the second coyote is as follows:

> "The second case was a juvenile male coyote (21 mo) found dead on a two-lane road, with minor evidence of skin tearing over the ventral neck and chest. Necropsy findings indicated additional moderate tearing of the muscle in the region overlying the thoracic inlet, although injuries did not penetrate the chest cavity. The chest was filled with blood. The interpretation for cause of death was severe acute hemorrhage, disproportionate to the mild to moderate trauma received from being hit by a vehicle. We suspected rodenticide toxicosis, and the liver was positive for brodifacoum and bromadiolone."

While it is possible that exposure to ARs was a contributing factor in the death of this coyote, it is unclear if this coyote would have recovered if it had not been hit by a vehicle. Typically, institutions such as the California Animal Health and Food Safety (CAHFS) lab at the University of California, Davis, require "antemortem or postmortem evidence of coagulopathy unrelated to another identifiable cause of hemorrhage (e.g., trauma)" combined with the detection of one or more AR compounds in the liver or blood of an animal in order to make a diagnosis of AR intoxication (CAHFS, 2015). The study authors did not follow this protocol because the hemorrhage noted in both coyotes was associated with "another identifiable cause of hemorrhage" (e.g., a puncture wound or getting hit by a vehicle). In both these cases, the study authors did not explicitly state that exposure to ARs was the cause of death, only that they were a contributing factor. However, they did not define "contributing factor" and there is no way to know if the puncture wound or the vehicular strike would have been sufficient to kill these coyotes if they had not been exposed to rodenticides.

Of the nine coyotes that were not tested for AR exposure, five were determined to have died due to vehicular collisions, one was determined to have died from a gunshot wound, one was killed due to "conflict resolution" at the Denver International Airport, and the causes of death for the last two coyotes were not determined. The study authors state that "The exposure of all five tested coyotes to rodenticides, especially brodifacoum, indicates the ubiquity of these toxicants in the urban landscape and their ability to reach higher levels in the food chain…" but this statement is not supported by the data because the selection procedure used to decide which animals to test was biased towards choosing those coyotes that were suspected of being exposed. Rather, the data shows that a total of 36% (5 of 14) of the coyotes that died during the study were determined to have been exposed to ARs. Alternatively, only 15% (5 of 33) of the collared coyotes included in the study tested positive for AR exposure. A sixth coyote that had been found in a rural area in Colorado was also tested because that coyote showed signs of hemorrhage. The study authors stated that they "found no evidence of any rodenticides in the liver, indicating that rodenticide toxicosis may not always occur in coyotes." The study authors go on to compare liver concentrations to acute oral LD_{50} values: "The acute oral LD_{50} value of bromadiolone in dogs ranges from 11,000 ppb to 15,000 ppb (Stone et al. 1999); the value in our study animal was 885 ppb." The validity of the comparison is questionable because an LD_{50} value is a dose (e.g., mg of active ingredient/kg of body weight of the animal receiving the dose), not a concentration (ppb or µg of active ingredient/kg of media [soil, food, liver, etc.]), and because the dose an animal ingests may not be comparable to the concentration detected in the liver when the time between exposure and testing (of the liver tissue) is unknown. This study contains some useful information because it provides an additional line of evidence that brodifacoum is detected more often than other rodenticides in the livers of non-target wildlife. However, the small sample size, the biased selection procedure, and criteria for diagnosis that is not in line with reputable necropsy labs reduces the validity of the study.

- **Serieys, L.E.K., Armenta, T.C., Moriarty, J.G., Boydston, E.E., Lyren, L.M., Poppenga, R.H., Crooks, K.R., Wayne, R.K., and Riley, S.P.D., 2015, Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study, Ecotoxicology, 24:844–862, DOI 10.1007/s10646-015-1429-5.**

This study compared AR exposure rates among bobcat (*Lynx rufus*) populations residing in two geographic areas near Los Angeles: 1) the Santa Monica Mountains National Recreation Area (SMM), and 2) public nature reserves and the Santa Ana Mountains in Orange County. AR exposure was evaluated from 1997-2012 in SMM and from 2006-2010 in Orange County. Liver samples were collected from bobcats that died in wildlife rehabilitation centers or from opportunistically found bobcat carcasses. Blood samples were collected from trapped bobcats, the majority of which were caught during the wet season, from mid-October to mid-February. Visual inspections were conducted on all bobcats for clinical signs of notoedric mange and skin scraping samples were collected to identify species of mites. Age class (greater than or less than two years), sex, weight, and various morphological measurements (e.g., body length, head circumference, etc.) were recorded for bobcats that were trapped and had blood samples collected. Necropsies were conducted on these bobcats to determine cause of death (when possible). These bobcats' specific ages were determined using the cementum annuli aging technique on an upper canine tooth in addition to the same parameters that were recorded for

trapped bobcats. Specific locations where bobcats were trapped or found dead were noted for all bobcats used in the study.

The AR screen analyzed blood, serum, and liver samples for warfarin, coumachlor, bromadiolone, brodifacoum, diphacinone, chlorophacinone, and difethialone. It is unclear why the FGAR coumachlor was included in the screen because it has never been registered in the United States. Additionally, the screen omitted difenacoum, which is a SGAR that is registered for use in California. Limits of Quantitation (LOQs) for liver samples were 10 µg/kg for brodifacoum, 50 µg/kg for bromadiolone, warfarin, and coumachlor, and 250 µg/kg for chlorophacinone, diphacinone, and difethialone. The study authors refer to these values as Limits of Detection (LODs) in the caption for their Figure 3, so it is unclear if these values represent LODs or LOQs. Blood samples had lower LOQs than liver samples, with an LOQ of 1 µg/kg for all analytes and LODs ranging from 0.28-0.45 µg/kg; the study authors did not specify which LOD went with which AR compound. Overall, 206 blood samples and 172 liver samples collected from wild bobcats were analyzed for exposure to ARs. Additionally, blood and liver samples were obtained simultaneously from 20 individual bobcats (only blood or liver samples were collected for all others).

Anticoagulant rodenticides were detected in 88% of liver samples and 39% of blood samples in both locations combined (SMM and Orange County). Anticoagulant rodenticide elimination half-lives are generally much shorter in blood and plasma samples than in liver samples (U.S. EPA, 2004). The faster elimination half-lives mean that there is less of a window, post-exposure, when these compounds can be detected in blood. Despite the high exposure rates, only one bobcat was determined to have died directly as a result of AR exposure. Brodifacoum, bromadiolone, difethialone, and diphacinone were the most frequently detected compounds overall. Brodifacoum and bromadiolone were detected in approximately 80% of the liver samples tested, whereas diphacinone and difethialone were detected in approximately 40% and 30% of the liver samples tested. In contrast, diphacinone was detected in approximately 30% of blood samples, with brodifacoum and bromadiolone detected in approximately 10% of blood samples. Coumachlor was not detected in liver samples, but it was detected in at least one blood sample, which is strange because no products containing that active ingredient have ever been registered in California or the United States. The study authors performed various statistical analyses based on data they had collected over the course of the study. Such data included age, sex, season (wet vs. dry), spatial correlates (i.e., land use in each bobcats home range), diagnoses of notoedric mange, and mortality. These parameters were compared to exposure data to see if any of them could serve as potential predictors of exposure (e.g., to see if female bobcats are more likely to be exposed than males). The study authors stated that there was no significant association between exposure and age of the 66 bobcats that were aged using the cementum annuli aging technique. There was also no significant association between exposure and sex (n = 151 for liver samples; n = 193 for blood samples), nor between exposure rates of liver samples (n = 162) comparing wet vs. dry season. However, in blood samples the study authors detected a significant difference between seasons, with anticoagulant rodenticides detected in 55% of samples in the dry season compared to 32% during the wet season (n = 195).

Generalized linear models were used to examine associations between exposure and various land uses in home ranges (approximately 5 km^2 for males and 2-3 km^2 for females) surrounding the

locations where bobcats were found (or captured). Spatial correlates were broken into five broad classifications of land use in places where bobcats were captured or found dead. These were: 1) agriculture (e.g., orchards, horse ranches, vineyards),
2) commercial and industrial (e.g., schools, offices, water facilities), 3) residential (e.g., multifamily/commercial, high and low density single family), 4) altered open space (e.g., golf courses, cemeteries, other recreational), and 5) natural (i.e., undeveloped). The last category, undeveloped natural areas, comprised the majority of land in both the SMM study area (67%) and the Orange County study area (59%). Total residential (the sum of multifamily/commercial high-density + high-density single-family + low-density single-family) comprised 22% of the land in the SMM study area and 24% of the land in the Orange Country study area. Agriculture, commercial and industrial, and altered open space composed the remaining ~11% and ~17% of land in the SMM and Orange County study areas, respectively.

Average home ranges in both study areas combined were approximately 5.4 km^2 for male bobcats and 2.8 km^2 for female bobcats. The study authors referred to these home range areas as buffer zones and used circular areas surrounding where the bobcats were found or captured to analyze land use and exposure data to make associations between land use patterns in each bobcats surrounding buffer zone and the compounds those bobcats were exposed to. Based on concentrations in liver samples, there were positive associations between: altered open space (areas such as golf courses) and bromadiolone and brodifacoum; commercial and industrial areas and bromadiolone and diphacinone; office and retail areas and brodifacoum; and total residential areas and brodifacoum and diphacinone. The study authors ran many different statistical analyses on various exposure parameters, but the validity of combining first and SGARs into a single parameter of "total residues" or "total number of compounds detected" is questionable because the SGARs are more toxic and have longer hepatic half-lives than the FGARs. The study authors acknowledge this in the discussion section, stating that diphacinone "is considered to pose less risk to nontarget wildlife than the more toxic second-generation ARs." The study authors stated that diagnoses of severe notoedric mange were found to be positively associated with difethialone exposure, brodifacoum exposure, and brodifacoum concentration. In the case of severe notoedric mange, the study authors listed "brodifacoum exposure" separately from "brodifacoum concentration." They found other associations that were also statistically significant, but the validity of those associations is questionable because they combined all ARs together into one parameter (e.g., total number of compounds detected, total residues, etc.).

Overall, this study provides a line of evidence showing that bobcats in the Los Angeles area had high exposure rates to ARs from 1997-2012. The study authors stated that a mange outbreak led to a precipitous population decline among bobcats from 2002-2006. This population decline was sufficient to cause a genetic bottleneck, a severe population level adverse effect. However, this study does not provide any useful information as to the efficacy of DPRs regulations in terms of reducing SGAR exposure rates among non-target wildlife. The study authors conclude this paper by stating that "measures that address residential use of ARs may be particularly effective in mitigating ecological risks associated with these compounds." DPR addressed this by enacting regulations in 2014 that made SGARs restricted materials, thereby taking them out of the hands of the general public and making them available only to certified pesticide applicators.

- **Gabriel, M.W., Diller, L.V., Dumbacher, J.P., Wenger, G.M., Higley, J.M., Poppenga, R.H., and Mendia, S., 2017, Exposure to rodenticides in Northern Spotted and Barred Owls on remote forest lands in northwestern California: evidence of food web contamination, Avian Conservation and Ecology 13(1):2. https://doi.org/10.5751/ACE-01134-130102**

This study examined AR exposure rates of two owl species in Del Norte, Humboldt, Western Trinity, and Northern Mendocino Counties in Northern California. This region is known for having many illegal cannabis cultivation sites. The barred owl (*Strix varia*) is considered a major threat to the viability of the threatened northern spotted owl (*Strix occidentalis caurina*) because it can outcompete them for resources and has been expanding its range into their critical habitat (as defined by the federal Endangered Species Act; https://www.fws.gov/southeast/endangered-species-act/critical-habitat/). Because of this, resource managers in California have decided to kill barred owls that reside in northern spotted owl critical habitat to improve the species chances of survival. This has provided the study authors with a rare opportunity to collect many barred owl liver tissue samples for AR testing. Northern spotted owls are federally listed endangered species, so only opportunistic sampling was conducted (i.e., carcasses found dead in the field).

Northern spotted owl livers were tested for ARs and carcasses were submitted for necropsy when they were in acceptable post-mortem condition. Rodents in the study area were also sampled and their livers were tested for ARs. Owl and rodent livers were tested for warfarin, diphacinone, chlorophacinone, coumachlor (never registered in the United States), brodifacoum, bromadiolone, difethialone, and difenacoum. The LOQ was 20 ng/g for all analytes except brodifacoum. The LOQ for brodifacoum was 50 ng/g. The livers of ten northern spotted owls were tested and seven of them were determined to be exposed to ARs. Brodifacoum was detected in all seven livers and bromadiolone was also detected in two of the seven livers (i.e., two owls were exposed to both brodifacoum and bromadiolone). The cause of death was identified for six northern spotted owls: three were killed by automobile strikes, two were due to emaciation following some unidentified infections, and one was killed by an unidentified predator. The livers of 84 barred owls were tested and 34 (40%) of them were determined to be exposed to ARs. Of those 34 barred owls, 27 were exposed to brodifacoum alone, three were exposed to bromadiolone alone, and four were exposed to both brodifacoum and bromadiolone. All of the bromadiolone detections were below LOQ. The study authors stated that six of the barred owls that tested positive for brodifacoum were above the LOQ with a range of 17-110 ng/g, but they also stated that the LOQ for brodifacoum was 50 ng/g, so it is unclear why a concentration of 17 ng/g would be included as a quantifiable level.

The study authors speculated that the lower exposure rates in barred owls may be due to their generalist dietary tendencies: whereas northern spotted owls consume rodents and lagomorphs as 81-96% of their diet, barred owls consume rodents and lagomorphs as 60-70% of their diet, with birds, insects, amphibians, reptiles, fish, snails, and crayfish making up a higher proportion of barred owl diets compared to northern spotted owls. It is unclear how the exposure rate for northern spotted owls was affected by the small sample size (n = 10) in comparison to barred owls (n = 84). A larger sample size would be more representative of the population and it is possible that a larger sample of northern spotted owls would have resulted in higher or lower

exposure rates for that species. However, the difficulties in acquiring additional samples of this protected endangered species in such a remote area are understandable.

The study authors also collected and tested livers from 18 Douglas squirrels (*Tamiasciurus douglasii*), 15 chipmunks (*Tamias* sp.), two northern flying squirrels (*Glaucomys sabrinus*), and two dusky-footed woodrats (*Neotoma fuscipes*). Anticoagulant rodenticides were not detected in any rodent livers. The study authors stated that the lack of anticoagulant rodenticide detections in rodents is not unexpected because rodents normally die within a few days of exposure.

The study authors point out that there are no legal uses for SGARs in the habitats where the owls in this study were collected and go on to state that "The use of not only the ARs *(anticoagulant rodenticides)* brodifacoum or bromadiolone, but other first and second-generation ARs, in addition to neurotoxicant rodenticides like bromethalin, have been documented in large quantities (10–90 lbs. per cultivation site) at numerous illegal marijuana cultivation sites where these owls were collected..." It should be noted that the only rodenticide active ingredients (anticoagulant or otherwise) detected in the owls tested in this study were brodifacoum and bromadiolone. Overall, this study provides another line of evidence that more non-target wildlife are exposed to brodifacoum than to any other rodenticide active ingredient. Of the 94 total owls tested in this study, 38 (40%) were exposed to brodifacoum, and nine (10%) were exposed to bromadiolone. The exposure rates reported in this study are high, especially considering that this is a remote densely forested region, with no nearby urban areas, where there are no legal uses of SGARs. Additionally, this study provides another line of evidence showing that brodifacoum has higher frequency of detections compared to other ARs.

- **Serieys, L.E.K., Lea, A.J., Epeldegui, M., Armenta, T.C., Moriarty, J., VandeWoude, S., Carver, S., Foley, J., Wayne, R.K., Riley, S.P.D., and Uittenbogaart, C.H., 2018, Urbanization and Anticoagulant Poisons Promote Immune Dysfunction in Bobcats, Proceedings of the Royal Society B, 285: 20172533. http://dx.doi.org/10.1098/rspb.2017.2533**

This study focused on various immunological parameters in blood samples collected from 124 bobcats in and around the Santa Monica Mountains National Recreation Area. Samples were collected from 2007 to 2012 and, in addition to blood samples, each bobcat was sexed, measured, and assigned an age class (juvenile or adult). The study authors measured 65 total measures of immune or organ function (henceforth "health parameters" [e.g., complete blood cell counts, serum chemistry, circulating cytokine levels, total T lymphocytes, etc.]). The study authors stated that there are no reference values for many of the parameters analyzed because, to their knowledge, no one has conducted these types of analyses on bobcats. Individual bobcats were tested for exposure to various pathogens and parasites including, but not limited to *Bartonella* spp., *Mycoplasma* spp., *Toxoplasma gondii*, feline immunodeficiency virus, and feline herpesvirus. All bobcats were inspected for signs of mange and four bobcats were excluded from the study because they were determined to have mange. The study authors did not want the immune response to mange to introduce noise into the dataset because this would complicate efforts to isolate the effects of anticoagulant exposure on immune system functions. Whole blood or serum samples were also analyzed for the presence of ARs. The AR analysis that the study authors used to determine exposure included warfarin, diphacinone, chlorophacinone,

coumachlor, bromadiolone, brodifacoum, and difethialone. It is important to note that coumachlor has never been registered for sale or use in the United States, and that the AR analysis did not include difenacoum, which is a SGAR that is registered for use in California. Urbanization was quantified for each individual bobcat as described in Serieys et al. (2015; reviewed above).

The three primary objectives of the study were: 1) to identify parameters indicative of immune impairment or cellular damage in organs that correlate with urban proximity or AR exposure; 2) to look for a predictable relationship between AR exposure and health parameters in a way that would allow analysis of the potential health parameter to be indicative of AR exposure; and 3) to describe a mechanism that could influence the susceptibility of bobcats living near urban environments to mange. The study authors identified three covariates (age class, *Mycoplasma haemominutum* infection, and *Bartonella* sp. exposure) which helped to explain significant variance in the top 20 (health parameter) principle components of the dataset. These three covariates were controlled for in further analyses. Next, the study authors looked for system wide associations between AR exposure and individual health parameters. A random forest classifier (an analytical method akin to a series of decision trees) was employed, which allowed them to use one analysis to evaluate the relative importance of all 65 health parameters simultaneously. The random forest method was used to complement linear models which were also used to look for associations between health parameters and AR exposure.

It is well established that the clearance time for AR residues is shorter in blood than in the liver; however, the way the study authors chose to frame this statement is somewhat misleading. The study authors stated that:

> "Testing blood for AR residues leads to 62% false negatives because blood measures only recent exposure [19]. We therefore hypothesized that (i) some individuals with no detectable levels of ARs in blood would be classified by the random forest as AR-exposed, and (ii) these individuals represent a set of truly AR-exposed individuals for whom the blood tests produced a false negative. If true, we would expect individuals living in more urbanized areas (where AR exposure is widespread) to fall into the misclassified group (i.e. to have immune profiles that are similar to known AR-exposed individuals, even though ARs were not detected in blood)."

This is confusing because the 62% false negative rate is not reported in the publication they cited (Serieys et al., 2015; reviewed above). Furthermore, the "62% false negative" rate can only be legitimately applied to the population of bobcats that they sampled during the timeframe when they were sampled. For example, the regulations making SGARs restricted materials went into effect in 2014, which is after the bobcats in Serieys et al. (2015) were sampled. If those regulations were successful in reducing exposure rates, then the 62% false negative figure could be much lower because reduced exposure rates would result in fewer negative detections in blood samples that would be labeled as false.

In another portion of the manuscript the study authors stated that

> "We previously documented that testing blood only indicates recent AR exposure events, thus leading to frequent false negatives (approximately 62% of the time; see [Serieys et al., 2015] for more detail) respective to an individual's history of exposure. Urbanization, therefore, is arguably a more sensitive measure of AR exposure than AR levels in the

tissues we are able to sample (i.e. peripheral tissues such as blood) [Serieys et al., 2015], but it can also reflect potential exposure to other toxicants from urban environments."

To say that urbanization "is arguably a more sensitive measure of AR exposure than AR levels in the tissues" is another statement that can potentially be misinterpreted.

The study authors concluded that:

> "Random forests revealed that the differences between AR-exposed and unexposed individuals were systemic and predictable such that the parameters themselves can be used to predict an individual's exposure status (predictive accuracy = 67.3%, error rate = 32.7% and AUC = 0.68, electronic supplementary material, figure S2a–b; proportion of individuals correctly classified as exposed and unexposed = 18/29 and 31/46)."

However, estimating the number of individual bobcats that are correctly classified as exposed or unexposed, could change due to regulations that went into effect in 2014. Those regulations made second-generation anticoagulant rodenticides restricted materials, and might have reduced exposure rates among bobcats, which in turn could change the rate of false negative detections in the blood of bobcats, which could change the random forest analysis prediction of false negatives. A predictive accuracy of 67.3% means that their predictions will be wrong 32.7% of the time, and it seems logical that the predictive accuracy could change in line with the ways in which rodenticides are used (i.e., changes in use patterns intended to reduce non-target wildlife exposure), and with changes in the quantity of ARs sold and used. This study provides a qualitative line of evidence that there are many health parameters that are affected by exposure to ARs.

- **Franklin, A.B., Carlson, P.C., Rex, A., Rockweit, J.T., Garza, D., Culhane, E., Volker, S.F., Dusek, R.J., Shearn-Bochsler, V.I., Gabriel, M.W., Horak, K.E., 2018, Grass is not always greener: rodenticide exposure of a threatened species near marijuana growing operations, BioMed Central Research Notes, 11:94, https://doi.org/10.1186/s13104-018-3206-z**

This is a research note, rather than a full study. It focused on a female northern spotted owl (*Strix occidentalis caurina*) that was found dead in 2017. The study authors estimated that this owl died less than 24 hours before they found it because "(1) the carcass was fresh with the eyes not sunken, (2) there were no fly larvae on the carcass, and (3) the male owl attempted to deliver a mouse to the carcass for ~ 5 min." The study authors stated that they had conducted 9,216 surveys since 1985 and this was the first time they had discovered a recently deceased northern spotted owl. The owl was necropsied and samples of blood and liver tissue were tested for rodenticide exposure. Specifically, the blood and liver samples were tested for the ARs coumafuryl, coumatetralyl, pindone, warfarin, coumachlor, diphacinone, chlorophacinone, bromadiolone, difenacoum, brodifacoum, difethialone, as well as for desmethyl-bromethalin, a metabolite of the neurotoxicant rodenticide bromethalin (the metabolite of the neurotoxicant bromethalin). Brodifacoum was detected in both samples (33.3-36.3 ng/g in the liver and <LOD-0.54 ng/mL in the blood; LOD for analysis in blood = 0.45 ng/mL). No other rodenticides were detected.

The owl was emaciated and had a heavy parasite load "with large numbers of *Leucocytozoon* spp. protozoa in red blood cells and *Elmeria* spp., coccidia and *Capillariid* spp. in the intestine."

There were no signs of trauma and tests for avian influenza virus, West Nile virus, and exposure to lead were all negative. Cholinesterase levels were normal, indicating no exposure to organophosphate or carbamate pesticides. The study authors concluded that the cause of death was emaciation and parasitism. The study authors stated that brodifacoum was not the primary cause of death because there was no internal hemorrhage, which would be symptomatic of AR intoxication. However, they also stated that "brodifacoum may have been an additional contributor to the owl's death."

There were seven active cannabis growing operations within 1.5 km of where this owl was found. The study authors described one illegal cannabis growing operation located 450 m from where this owl was found. Although that operation was shut down in 2015, there was 23 kg of brodifacoum laced bait around its perimeter, providing evidence that many of these illegal cannabis grow operations are using pesticides illegally (i.e., not in compliance with the labeled uses). The study authors hypothesized that dusky-footed woodrats (*Neotoma fuscipes*) are the mechanism of transmission of ARs from illegal marijuana grow operations to higher trophic levels. This is because woodrats are often abundant in forest clearings such as those created by fire and logging. Illegal cannabis growing operations clear out the forests in similar ways to allow light to reach the cannabis plants. Additionally, woodrats are known to use plants with high monoterpene content (such as marijuana and California bay) as nest material because they can act as insect larvicides. The forest clearings also create increased edge, which is where northern spotted owls often forage. Overall, these illegal cannabis grow operations are creating habitat that attracts both woodrats and owls, so when ARs are available for woodrats to consume, the potential exists for them to be transferred up the food chain. This study presents an additional line of evidence that illegal uses of pesticides in illegal cannabis grow operations are contaminating food webs and impacting threatened species in remote forested areas of California where the SGARs have no legal uses.

- **Fraser, D., Mouton, A., Serieys, L.E.K., Cole, S., Carver, S., Vandewoude, S., Lappin, M., Riley, S.P.D., Wayne, R., 2018, Genome-wide expression reveals multiple systemic effects associated with detection of anticoagulant poisons in bobcats (*Lynx rufus*), Molecular Ecology, 00:1–18, https://doi.org/10.1111/mec.14531**

This study examined various sublethal effects of rodenticide exposure using 52 blood samples collected from bobcats captured in the Simi Hills, Hollywood Hills, and the Santa Monica Mountains from 2008 to 2012. Twenty-six of the blood samples were from bobcats that had been exposed to ARs and 26 of the blood samples were from bobcats that had not been exposed to ARs. The samples were also balanced in terms of age and sex. The AR screen tested for brodifacoum, bromadiolone, difethialone, diphacinone, warfarin, chlorophacinone, and coumachlor. It should also be noted that coumachlor has never been registered for use in California. Additionally, the screen did not include difenacoum, which is a SGAR that is registered for use in California. The bobcats from which these samples were collected did not appear to have any signs of disease.

Serum samples were analyzed for various viral and bacterial pathogens. Total RNA was extracted from whole blood samples, then quantified and sequenced. The genome from the domestic cat (*Felis catus*) was used as a reference genome. The study authors conducted various

statistical analyses (e.g., principle components analysis, linear regression, etc.) and found that there were 1,783 genes that were significantly associated with exposure status. Of those, 530 were downregulated and 1,253 were upregulated. Among the genes that were downregulated were genes related to wound healing, epithelial integrity, white blood cell production, and several genes involved in the allergic response. Among the genes that were upregulated were genes that may lead to activation of the adaptive immune system and processes related to xenobiotic transformation. Overall, the study authors stated that "the up- and downregulation of numerous cytokines demonstrate a pronounced dysregulation of critical mediators of immune function, implying both immunosuppressive and stimulating effects of AR [anticoagulant rodenticide] exposure." Other genes that were downregulated in AR exposed bobcats suggested that exposure could influence epithelial maintenance and formation. The study authors stated that some of these genes could potentially help provide an explanation as to the link between AR exposure and mange in bobcats. More specifically, the study authors stated that the association between AR exposure and genes related to immune regulation and epithelial integrity could predispose bobcats to opportunistic infection by mange causing parasites. Furthermore, the cumulative effects that interfere with the regulation of cellular functions related to AR exposure likely inhibit the healing of wounds, allowing for mange lesions to grow, which can ultimately lead to death. Overall, this study identifies several pathways through which exposure to ARs can lead to effects that decrease the fitness of bobcats and can lead to population level effects.

The following publication was submitted by Mr. Graf. DPR scientists evaluated and analyzed this publication. A summary is presented below.

- **Novak, K., Torfeh, D., 2017, Raptor Pilot Study for Levee Protection - Integrated Pest Management Program, Ventura County Public Works Agency, Watershed Protection District, available via: <https://vcportal.ventura.org/BOS/District2/RaptorPilotStudy.pdf>, accessed October 16, 2018.**

This study was not peer-reviewed and many of the statements and claims in this study are not supported by citations. The purpose of this study was to quantify and compare the efficacy of raptors in reducing ground squirrel populations in comparison to FGARs. Burrow damage caused by gophers was also quantified, but the FGAR bait used on the levees is not labeled for gophers, so ground squirrels were the main focus of the study.

A baseline was established before the start of the study by finding and filling all ground squirrel burrows in the study area with a cement bentonite grout. The amount of grout used was equal to the volume of two cement trucks (4,400 gallons of grout in a 2.56 mile stretch). There were two phases: Phase 1 compared two 6,000 foot reaches of the levee that runs along Revolon Slough in Oxnard, CA. During Phase 1, the first reach was called the raptor test site and the second reach was called the control site. The two reaches were separated by a 3,000 foot buffer zone. In the raptor test site, AR bait stations were removed and replaced with raptor perches. In the control site, diphacinone bait was applied using rodenticide bait stations. The study authors monitored the perches, and quantified new rodent burrows, burrow grouting, rodenticide consumption, raptor sightings, agricultural use in adjacent fields, as well as an analysis of scat and raptor pellet contents (undigested materials, such as hair and bones, regurgitated by the raptors). Monitoring

was conducted by five individuals on each reach during alternating weeks (control site one week, then the raptor site the next week). Additionally, the contents of the raptor pellets were analyzed to determine what the raptors were feeding upon. The study authors noted that the crops grown in adjacent fields were impacting the efficacy of the bait stations because ground squirrels have a preference for some crops, such as berries, over diphacinone treated grains. This motivated the study authors to develop a second phase for the study. During Phase 2, the control site was renamed as the "modified control site" and the rodenticide bait stations were replaced with raptor perches at that site.

The crops grown in adjacent fields were similar during the two phases of the study, but there was more fallow land in 2017, compared to 2016. The study authors stated that fewer annual crops in 2017 could result in fewer squirrels. The study authors tallied raptor observations during 65 monitoring outings from April 2016 to August 2017. Red-tailed hawks had the most observations (101), but the study authors estimated that the same three to four hawks were observed repeatedly. White tailed kites were the next most common, with 27 observations, followed by Cooper's hawks (20 observations), ospreys (10 observations), and northern harriers (8 observations). Red-shouldered hawks, peregrine falcons, merlins, and burrowing owls were all observed three times each. Great horned owls were observed twice and there was one observation of a Swainson's hawk. Barn owls were not observed, but raptor pellet analysis indicated that barn owls and great horned owls were hunting gophers during the study. The presence of scat revealed that the perches were being used by raptors soon after installation. During Phase 1, from April to November of 2016, there was a 66% reduction in new ground squirrel burrows on a per mile, per month basis in the raptor site compared to the control site. When October and November were excluded from the 2016 analysis, there was a 57% reduction in new ground squirrel burrows on a per mile, per month basis in the raptor site compared to the control site. When the control site during Phase 1 was compared to the modified control site during Phase 2, there was a 47% reduction in new ground squirrel burrows on a per mile, per month basis (Table 3). It is unclear why the study authors decided to exclude September, October, and November from Phase 2. In the control site, those three months accounted for more new squirrel burrows than the period from April to August of 2016. There were 206 observed new squirrel burrows in the control site from April to August of 2016, and 224 observed new squirrel burrows in the control site from September to November of 2016. This presents some uncertainty as to the results, because it is unclear how the comparison between the control site during Phase 1 and the modified control site during Phase 2 would have been different if September, October, and November had been included in the analysis. The study authors did not provide an explanation as to why the months with the most new squirrel burrows were excluded from Phase 2.

The study authors only reported burrow grouting for the entire study area, and did not distinguish between the control site, the raptor site, or the 3,000 foot buffer zone separating the two sites. During Phase 1, new burrows were grouted eight times after the additional baseline grouting and a total of 1,400 gallons of grout was injected into the levees. During Phase 2, a total of 700 gallons of grout was injected into the levees during six grouting operations from March 3rd to August 27th of 2017. Although anecdotal, the grouting crews reported that there were fewer burrows in 2017 and the burrows that were grouted had less penetration into the levees. An independent contractor was used for rodenticide applications. They made weekly inspections and

applied oats infused with diphacinone at 0.005% into bait stations as needed. The study authors reported that a total of 84.5 pounds of bait was consumed during Phase 1. The contractor who applied the rodenticide also reported to the study authors that consumption of rodenticide bait increased after raspberries were harvested adjacent to the control site.

A total of 107 raptor pellets were analyzed to determine which raptor species were hunting in the area and what the raptors were feeding upon. Of the pellets analyzed, 49% were from owls and 51% were from hawks or other non-owl raptors. The study authors discussed which target species were found in the raptor pellets in the text of the report, and even provided a table, but they did not mention any impacts on non-target wildlife in the text of the report. However, Appendix F on Page 52 of their report contains raw data for the raptor pellet analysis which shows that the raptors were consuming many non-target wildlife. Ground squirrels were the focus of the study and the raptor pellet analysis found a minimum of nine ground squirrels. However, a minimum of 18 American coots and 18 passerine species were also found in the raptor pellets and/or raptor scat, suggesting the raptors were killing twice as many non-target birds as ground squirrels. Additionally, the raptor pellet analysis showed that raptors were also feeding on frogs (e.g., *Pseudacris* sp., African clawed frog, *Rana* sp.), snakes (e.g., gopher snake), lizards, other reptile species, crabs (e.g., kelp crab), crayfish, other bird species (e.g., Virginia rail, red-winged blackbird, Eurasian collared dove, song sparrow), lepidopteran larvae, as well as a variety of mammals and terrestrial invertebrates. Many of the non-target wildlife species found in raptor pellets would most likely not have been exposed to or affected by ARs (e.g., coots, blackbirds, sparrows, frogs, lizards), so there is a trade-off in impacts to non-target wildlife that the study authors did not discuss in the text of the report.

This study was not replicated. However, Phase 2 allowed the study to continue into a second year with nearly identical agricultural conditions during both years in the raptor site, and the similarity of the results in the raptor site (15.7 new burrows/mile/month during Phase 1 and 15.8 new burrows/mile/month during Phase 2) increase confidence in the results (Table 3). The study authors stated that "neither method has completely eliminated burrows" and that "regular inspection and burrow grouting are critical elements" that must continue to determine whether rodenticides or raptors have greater efficacy at controlling populations of burrowing rodents. The study authors created a criteria for expanding the program. They stated that "earthen facilities that have natural areas on adjacent properties" would be appropriate candidates for expansion of the raptor program, but that urban areas would not be good candidates for raptor perches. Overall, this study showed that the installation of raptor perches and nesting boxes can be more effective than rodenticides under certain conditions.

Table 3 – New ground squirrel burrows per mile per month during the Raptor Pilot Study for Levee Protection. In the raptor test site, rodenticide bait stations were removed and replaced with raptor perches. The control site used rodenticide bait stations without raptor perches. In 2017, the control site was renamed the modified control site because the rodenticide bait stations were removed and replaced with raptor perches.

Table 3. New Ground Squirrel Burrows (new burrows per mile per month) *	
Phase 1 (April to November 2016)	
Raptor Test Site	16.0
Control Site	47.3
Percent reduction in burrows	*66.2%*
Phase 1 (April to August 2016)	
Raptor Test Site	15.7
Control Site	36.3
Percent reduction in burrows	*56.7%*
Phase 2 (April to August 2017)	
Raptor Test Site	15.8
Modified Control Site	19.4
*Percent reduction in burrows **	*46.6%*
* This table was reproduced and modified from Novak and Torfeh (2017). ** Percent reduction when comparing the control site during Phase 1 (from April to August of 2016) to the modified control site during Phase 2 (from April to August of 2017). The study authors did not explain why Phase 2 ended in August, rather than November.	

- **Emails from Drs. Seth Riley and Laurel Serieys to Jan Dougall (Las Virgenes Municipal Water District), Kian Schulman (Poison Free Malibu), and other National Park Service staff**

These emails, submitted by Mr. Graf, discuss research and opinions about ARs in response to an inquiry from a concerned citizen. The emails do not provide scientific data.

- **Letter from Allen M. Fish, Director, Golden Gate Raptor Observatory**

A letter from Allen M. Fish was submitted to DPR by Michael Graf. The letter does not provide any additional scientific data.

- **Table contained in Mr. Graf's letter**

This table contains numbers without any units and was provided to DPR without any explanation of what these numbers represent, how they were generated, or if the methods used to generate these numbers are scientifically sound. As a result, it cannot be evaluated or used to make regulatory decisions. Raw data is also required so that DPR scientists can conduct independent calculations and reproduce the numbers in the table.

Table 4

	Pre-Regs	Year 1	POST
brodifacoum	94.	78.	89.
bromadiolone	59.	52.	69.
difethiolone	10.	28.	34.
difenacoum	1.5	7.4	0.
diphacinone	13.	50.	47.
chlorophacinone	4.4	11.	9.6
warfarin	1.5	5.6	6.1
Total Cases	68	54	114
Bromethalin Cases	0	3	7

Summaries of AR Data and Information from Regulatory Agencies

- **A Summary of Studies Described in a U.S. EPA Risk Assessment**

The U.S. EPA (2004) compared risks to non-target birds in a review of secondary toxicity studies. In some of the studies they reviewed, prey (mostly rats or mice) were poisoned with rodenticides and their whole or ground carcasses were fed to birds (raptors and scavengers). The review noted 42% mortality (63 of 149 individual birds) in 11 studies in which birds were fed brodifacoum-poisoned prey. In contrast, five studies conducted with bromadiolone resulted in 8% mortality (9 of 118 individual birds) when birds were fed bromadiolone-poisoned prey. Although not all these studies examined sublethal effects, surviving birds that were fed bromadiolone-poisoned prey exhibited fewer sublethal effects than surviving birds that were fed prey poisoned with brodifacoum. The U.S. EPA review also described two more studies in which barn owls were fed mice that had been poisoned with brodifacoum or bromadiolone. In those studies, four of six owls fed brodifacoum-poisoned mice died, but all six of the owls fed bromadiolone-poisoned mice survived (U.S. EPA, 2004).

Another study described in the review compared secondary toxicity risks of three FGARs and three SGARs to barn owls. Six owls per test group were fed rats that had been offered nontoxic laboratory feed or baits laced with either brodifacoum (20 ppm), bromadiolone (50 ppm), or difenacoum (50 ppm). The rats were free to choose between the non-toxic laboratory feed or the

rodenticide-laced bait. The barn owls were exposed to these rats for ten days. After ten days of exposure, five of six owls fed rats exposed to brodifacoum were dead, one of six owls fed bromadiolone-exposed rats was dead, and all six of the owls fed difenacoum-exposed rats survived. It is important to note that owl mortality in the brodifacoum test group was higher despite the fact that the concentration of brodifacoum bait that the rats fed upon was lower than for the other two SGARs. In the same experiment, two owls per test group were exposed to rats fed either diphacinone (50 ppm), chlorophacinone (50 ppm), or fumarin (250 ppm; an FGAR never registered for use in California). There were no mortalities and no observed sublethal effects in any of the owls fed rats exposed to FGARs (U.S. EPA, 2004).

- **DPR Pesticide Sales and Use Reporting Data**

DPR tracks the sales and use of pesticides, including ARs. It is important to note pesticide use reporting data only includes pesticides used by professional applicators that have been licensed and certified by DPR. Sales data is reflective of pounds of pesticides sold as self-reported by registrants. However, the fact that a pesticide is sold in a given year is not necessarily reflective of its use.

DPR can then use the sales and use data to qualitatively compare exposure rates from different active ingredients to their sales (Figure 9) and use (Figure 10). For example, according to DPR's use and sales data more diphacinone was used/sold, with the exception of use of bromadiolone in 2016, than any of the other rodenticides. However, exposure rates for diphacinone are relatively low in comparison to other ARs.

There are some trends in the sales and use data. Specifically, diphacinone use increased from 2009 to 2013, then decreased back to 2009 levels in 2015 (Figure 9). Diphacinone, being a FGAR, was not affected by the 2014 regulations enacted by DPR, so it is unclear what is driving this trend. In contrast, sales of diphacinone declined from 2011 to 2014, then increased from 2014 to 2017 (Figure 10).

Bromadiolone use increased approximately three-fold from 2015 to 2016, then declined in 2017, but the increased use of bromadiolone is not reflected in the sales data (Figures 9 and 10). Brodifacoum use has always been relatively low compared to other ARs, because it is not favored by professional applicators (DPR, 2013). Brodifacoum sales have decreased since the 2014 regulations went into effect, from 34.5 pounds of active ingredient in 2013, to a low of 3.5 pounds in 2015, and have increased slightly since then to 5.7 pounds in 2017 (Figure 10). Based on the limited data on file, DPR determined that decreased sales of brodifacoum do not appear to have led to decreased exposure rates among non-target wildlife.

Figure 9 – A summary of Pesticide Use Report data from 2005-2017. All certified applicators in California are required to submit pesticide use reports to county agricultural commissioners, who in turn, report to DPR. This chart displays AR use by professional certified applicators, not the general public. Certified applicators report use to County Agricultural Commissioners, who report to DPR. Therefore, DPR cannot attest to accuracy of the values used to generate this graph.

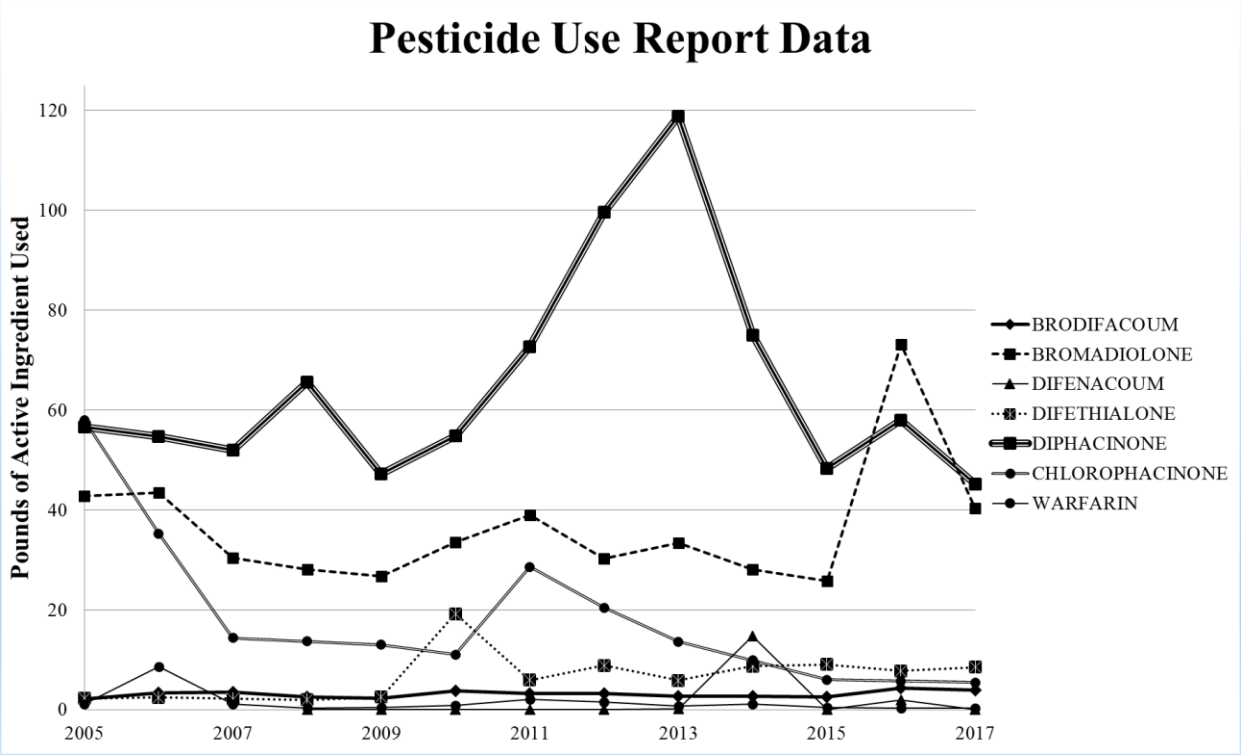
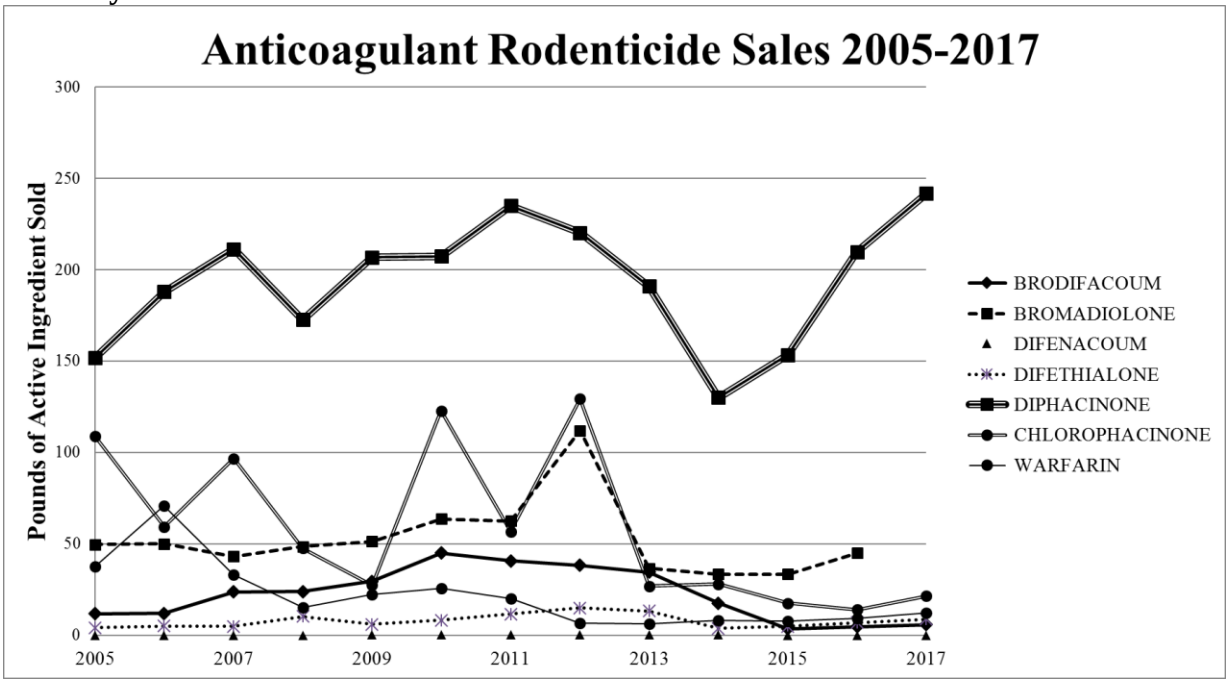


Figure 10 – A summary of AR sales data from 2005-2017. Sales data for bromadiolone in 2017 indicated that 638 pounds of active ingredient was sold. This is most likely an error, so 2017 sales data for bromadiolone is not present in this graph. DPR sales reports are based on information obtained from a system of self-reporting, so DPR cannot attest to the accuracy of the data.



Conclusion

As evidenced by its mission statement, DPR is guided by the principle that pesticide use should not cause unacceptable risks to human health or the environment. California law (Food and Agricultural Code 12824) requires DPR to "eliminate from use in the state" any pesticide that "endangers the agricultural or nonagricultural environment, is not beneficial for the purposes for which it is sold, or is misrepresented." To fulfill this mandate, DPR is required to enact "continuous evaluation" of currently registered pesticides. Multiple programs are set in place for this goal, including DPR's formal Reevaluation Program. Given evidence that the use of a pesticide may be causing significant adverse effects to people or the environment, DPR is required to investigate. If the Director finds from the investigation that a significant adverse impact has occurred or is likely to occur, DPR is required to reevaluate the pesticide and determine if it should remain registered or if additional mitigation measures are needed.

Risk is the combination of hazard and exposure. When evaluating a pesticide's risk to non-target organisms, toxicity, persistence, and bioaccumulation are the three main factors that should be considered. These three factors stem from inherent physicochemical parameters of a molecule that cannot be changed and are determined through laboratory testing. They are controlled by the interaction, on a molecular level, between the active ingredients and the biological receptors in target and non-target organisms. In addition, the way that a pesticide product is used (i.e., the use patterns) also affects its risk to non-target organisms. Use patterns can be changed by modifying the directions for use and/or by adding additional restrictions (e.g., only allowing use in or near

structures such as houses). In this case, DPR is investigating the risk of non-target wildlife exposure to anticoagulant rodenticides.

The data currently on file with DPR provide no basis for placing FGARs into reevaluation. First, the physicochemical properties of the FGARs are less toxic (Table 1), less persistent (Table 2), and less bioaccumulative (Table 3) than the SGARs, demonstrating that the inherent risk of the FGARs is lower. Second, the exposure rates among non-target animals are lower for FGARs than for SGARs (Figures 1, 3, 6, 7, and 8). For example, U.S. EPA (2004) observed that owls that were fed rats exposed to FGARs showed no mortalities and no observed sublethal effects. Finally, there is a general downward trend in FGAR exposure rates (Figure 3). As a result, DPR finds that current uses of FGARs are unlikely to have a significant adverse impact to non-target wildlife.

Compared to FGARs, SGARs are all more toxic, more persistent, and more bioaccumulative. Several of the publications submitted by Graf provide lines of evidence showing that there have been population-level adverse effects among bobcats in Southern California due to exposure to SGARs. Of particular note is Serieys et al. (2015), which found statistically significant associations between SGARs and mange, but not between FGARs and mange. These sublethal effects can affect fitness and have population level effects (Serieys et al., 2015). A severe outbreak of mange from 2002 to 2006 caused a genetic bottleneck among bobcats in Southern California (Serieys et al., 2015) which may be irreversible. Though available data is extremely limited and the true extent of exposure is unknown, it is possible that other predatory/scavenger species may also suffer similar significant adverse effects.

DPR enacted regulations in 2014 that were designed to reduce the risk of non-target wildlife exposure to SGARs. The regulations changed the use patterns, and restricted the purchase, sales, and use of second-generation ARs to certified applicators only. However, the limited data that DPR has on file shows that exposure rates have not decreased among SGARs (Figures 1, 2, and 8).

In addition, there is evidence to suggest that brodifacoum may have the highest level of risk within the SGARs. Brodifacoum consistently had higher exposure rates in non-target organisms than any other rodenticide that was disproportionate to its use: in the DFW mountain lion database; in the non-target organism loss reports submitted by DFW (compiled into a database and independently analyzed by DPR scientists); in the WildCare data that DPR already had on file (Part 4); and in the following peer-reviewed publications submitted by Graf: Vyas et al. (2017); Poessel et al. (2015); Gabriel et al. (2017); and Franklin et al. (2018). These lines of evidence indicate that more non-target organisms are exposed to brodifacoum than to any of the other ARs tested.

Collectively, the physiochemical properties of the SGARs, high exposure rates, and population-level impacts demonstrate that SGARs have a significant adverse impact to non-target wildlife.

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DPR Anticoagulant Rodenticide Informal Public Workshop

September 24, 2025

Overview

- Overview of Anticoagulant Rodenticides (ARs)
- High level proposed mitigation
- Current AR restrictions based on legislative action
- Details of draft proposal
- Comment Period
- Next Steps

Anticoagulant Rodenticides

Anticoagulant rodenticides prevent blood from clotting, leading to uncontrolled hemorrhaging and toxicosis.

- Second-generation anticoagulant rodenticides (SGARs)
  - **Brodifacoum**
  - **Bromadiolone**
  - **Difenacoum**
  - **Difethialone**
- First-generation anticoagulant rodenticides (FGARs)
  - Chlorophacinone
  - **Diphacinone**
  - Warfarin

***Bolded pesticides are under formal DPR reevaluation**

The proposed mitigations would mitigate all FGARs and SGARs as a holistic approach.

Mitigation: Reduce impacts to wildlife and maintain necessary uses of ARs

Reduce repeat exposure of non-target wildlife for all ARs

- Reduce overall amount in the environment
- Reduce how long they are available in the environment

Educate users on sustainable rodent management

- Education
- Sustainable Rodent Management Plan

How are we proposing to do this?

Propose regulations that:

- Classify all ARs **restricted materials.**
- Limit **where** ARs can be used to those that protect public health, agriculture, and water.
- Limit applications to a maximum of **35 consecutive days** at most sites with a maximum of 105 days annually per site for any AR.
- Require **training** on sustainable rodent management that incudes rodent biology and choosing the right tool for managing rodents.
- Require developing and maintaining a **sustainable rodent management plan** that addresses how the businesses or operators will approach rodent management decision making.

What is a Site?

Existing product labels specify the sites where a product can be used

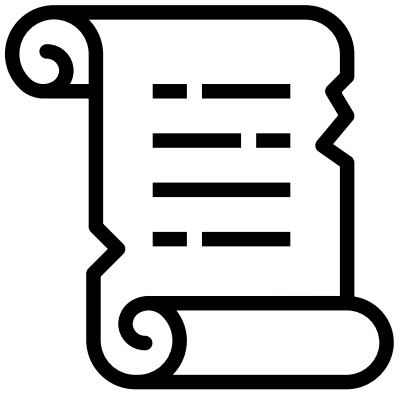
Proposed regulations would further restrict sites where ARs could be used

- Restricts use in and around man-made structures to within 50 ft of listed structures
- Specifies when use would be exempted from regulations and, in some cases, the sites where they would be exempt.

Legislation

Section 12978.7 of the California Food and Agricultural Code (FAC) contain use restrictions, considerations for reevaluation and concurrence requirements with the California Department of Fish and Wildlife (CDFW).

- 2020: **AB1788 -** Prohibits use of SGARs except at certain sites
- 2023: **AB1322 -** Prohibits use of diphacinone (FGAR) except at certain sites
- 2024: **AB 2552 -** Prohibits use of remaining FGARs (chlorophacinone and warfarin) except at certain sites



Current vs Proposed Restrictions

Current restrictions (FAC § 12978.7):

- Applications are only allowed by exempted users or at exempted use sites

Proposed restrictions:

- Specifies manmade structures where ARs can be used, via site definitions in statute
- Limits duration of use
- Requires applicator training and development of a Sustainable Rodent Management plan

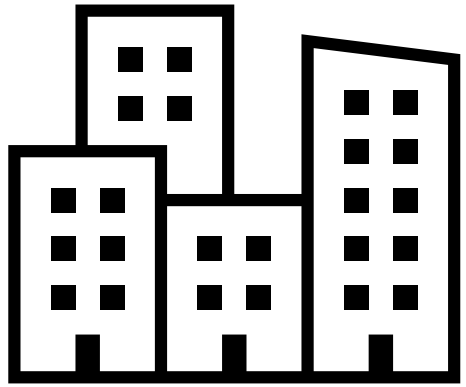
Allowed Use at Manmade Structures

Use at man-made sites is only allowed in listed sites

- Sites picked to protect public health
- Subject to the use duration restriction

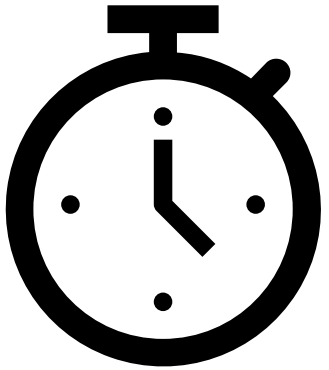
Use for public health, water supply, agriculture, protecting endangered species, and research that meet statutory definitions

- Exempt from duration restriction as specified



Limitation on Duration of Baiting

- **35 consecutive day** limit of any AR per application
- 2 additional 35-day applications permitted per year, for a cumulative annual total of **105 days** per site.



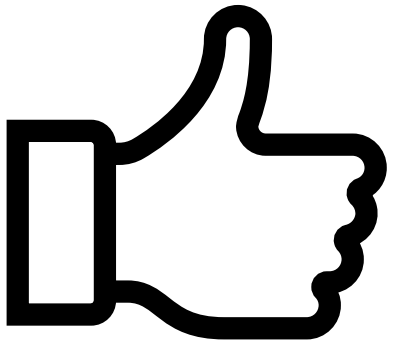
Basis:

- Registrant submitted data indicate that this timeframe is efficacious
- Studies have shown a 70% reduction in rodent populations in 35 days

Proposed Exemptions

The following uses **would be exempt** from the manmade structures and duration restrictions:

- Public health
  - As declared by State Public Health Officer
  - Use by vector control
- Nonnative invasive species eradication on offshore islands
- CDFW invasive rodent population eradication to protect endangered species/habitats
- To protect water and hydroelectric infrastructure
- FGAR use in agriculture
- Research for continuous evaluation



Holistic rodent management

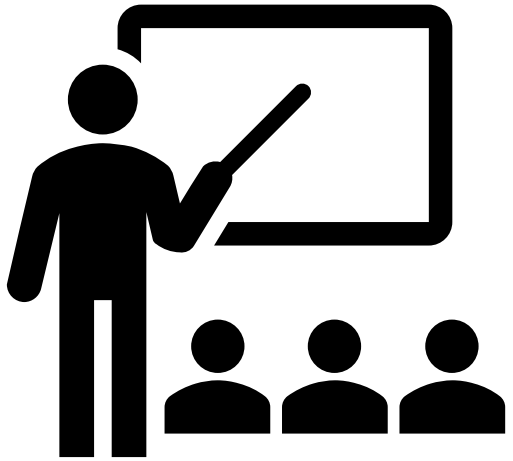
- Reduced use is critical to protecting non-target wildlife and will help ensure effective pest management critical to addressing rodent management more holistically.

- To support this, the draft mitigation includes a training requirement for AR applicators and development of a Sustainable Rodent Management plan for businesses and private applicators.

Sustainable Rodent Management Training

- **Proposed use requirement**: To use ARs individuals must take annual training to increase awareness and adoption of integrated pest management (IPM) practices, with record retention for two years.

- The course would include **Integrated Pest Management and Sustainable Pest Management principles** (as defined in the FAC sections 11401.7 and 11412).



Training Implementation Options

Outside of rulemaking, DPR is considering whether the training will:

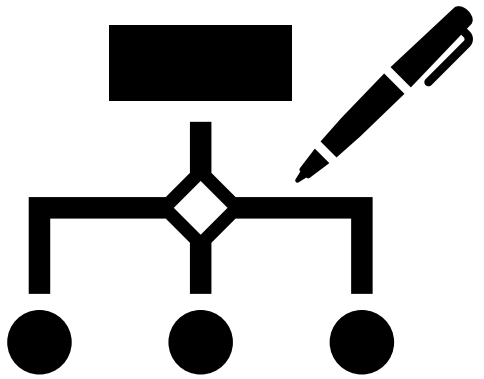
- DPR provided or DPR approved
- Count towards DPR and SPCB licensure (CE credits)

DPR is looking for public feedback on which of these options may be the best fit for implementing this training and proposed topics to include in the required training outline detailed in the regulation.

Sustainable Rodent Management Plan

Each business would be required to write, implement and retain records of a Sustainable Rodent Management plan.

- **General and is not required to be site-specific**
- Used as a **decision-making tool**, not a prescribed set of actions for every specific scenario.



Sustainable Rodent Management recordkeeping requirement:

- **Site-specific use records** kept at a central business location that tracks the dates ARs are deployed and collected by site to support compliance with the 35-day limit.

Thank you

California Department of Fish and Wildlife (CDFW)

California Department of Food and Agriculture (CDFA)

Structural Pest Control Board (SPCB)

California Department of Public Health (CDPH)

Where DPR wants feedback specifically

- Does the rulemaking text capture the intent of mitigation?
- Refinements to exempted sites
- Training topics and implementation options
- Site-specific use duration recordkeeping
- 12-month delay between effective date and training requirements

Next steps

- Draft proposed regulatory text are available on our website (www.cdpr.ca.gov).

- 45-day informal comment period
  - Please submit comments to DPR's Public Comment Portal at https://cdpr.commentinput.com?id=JsSRaG6NA by November 8, 2025.

  - Please submit clarifying questions to: Rodenticide.Comments@cdpr.ca.gov

SmartComment QR Code

Questions?

Northern spotted owl | Dave Roelofs, BLM

CROSSWALK OF ANTICOAGULANT LEGISLATION AND PROPOSED REGULATIONS

CURRENTLY ALLOWED USES (FAC 12978.7 AND ENF 24-20)	DPR 2025 PROPOSED REGULATIONS
Governmental agency employee for public health (g)(1)	Maintained with proposed 3CCR (d)(3) & (5)
Employee or contractor of a governmental agency or public utility to protect the water supply and hydroelectric energy (g)(2)	Maintained with proposed 3CCR (d)(4)
Mosquito or vector control district to protect the public health (g)(3)	Maintained with proposed 3CCR (d)(3) & (5)
Eradication of nonnative invasive species on offshore islands (g)(4)	Maintained with proposed 3CCR (d)(1)
Control or eradication of invasive rodents by CDFW to protect threatened or endangered species (g)(5)	Maintained with proposed 3CCR (d)(2)
Public health need as determined by State Public Health Officer (g)(6)	Maintained with proposed 3CCR (d)(3)
Research (g)(7)(8)	Maintained with proposed 3CCR (d)(7)
Medical waste generator as defined in HSC 117705 (h)(1)(A)	Maintained with proposed 3CCR (a)(1-5) with 35 consecutive day duration restrictions
Facility registered and inspected under Federal Food, Drug, and Cosmetic Act (h)(1)(B)	Maintained with proposed 3CCR (a)(6) with 35 consecutive day duration restrictions
Agricultural Activities (h)(2)	
Warehouse storing food for human or animal consumption	Maintained with proposed 3CCR (a)(7-10) with 35 consecutive day duration restriction in and around manmade structures, FGAR use away from manmade structures maintained.
Food production sites including slaughterhouse or cannery	
Factory	
Brewery, winery	
Ag production site housing water storage and conveyance	
Ag production site housing rights-of-way and transportation infrastructure	
Prohibited Uses Under Current Law	
Residential Use	Not Allowed unless it meets an exception
Restaurant (unless attached to a brewery or winery)	Maintained with proposed 3CCR (a)(8-9) with 35 consecutive day duration restriction, FGAR use away from manmade structures maintained
Grocery stores	Maintained with proposed 3CCR (a)(7) with 35 consecutive day duration restriction, FGAR use away from manmade structures maintained
Airports, offices, constructions sites, ports and terminal buildings, shipyards, lumber yards, schools, shopping malls unless identified in allowed uses	Use not allowed unless it meets an exception.
Non-production ag sites such as cemeteries, golf courses, parks, highways, and railroads	Use not allowed in or around manmade structures unless it meets one of the exceptions. FGARs can only be used away from manmade structures with the 35 consecutive day duration limit if allowed on the product label.
Wildlife habitat area - park or wildlife refuge managed by a state agency, regional government, quasi-government agency, or a special district	

Resource for pre-regulatory workshop on mitigation updated on Sept. 9, 2025.

DPR Draft Proposed Anticoagulant Rodenticide Regulation Text

DELIBERATIVE DRAFT

Key to Draft Regulatory Text: Black text is existing reg text, Blue text is new/added, Green text is moved, Red text are proposed deletions

Restricted Materials Regulations (CCR)

Subchapter 4 - Restricted Materials (Article 1 to 5) -

Article 1 - Restricted Materials (§ 6400 to 6402), § 6400 - Restricted Materials

The Director designates the pesticides listed in this section as restricted materials.

.....

(e) Certain other pesticides:

...

Carbofuran (Furadan)

Chlorophacinone

Chloropicrin

...

Difethialone

Diphacinone

Diphacinone sodium salt

Disulfoton (Di-Syston), except when labeled only for one or more of the following uses: home use, structural pest control, industrial use, institutional use, and use by public agency vector control districts pursuant to section 116180 of the Health and Safety Code.

...

Tributyltin, organotin, or a tri-organotin compound formulated as an antifouling paint, coating or compound and labeled for the control of fouling organisms in an aquatic environment.

Warfarin

Warfarin sodium salt

Zinc phosphide, except when labeled only for one or more of the following uses: home use, structural pest control, industrial use, institutional use, and use by public agency vector control districts pursuant to section 116180 of the Health and Safety Code

Article 2 - Possession and Use Limitations (§ 6404 to 6417)

§ 6414 - Permit Exemptions

.....

(h) No permit shall be required for products containing brodifacoum, bromadiolone, difenacoum, difethialone, chlorophacinone, diphacinone, diphacinone sodium salt, warfarin, or warfarin sodium salt, unless otherwise required by the commissioner.

DPR Draft Proposed Anticoagulant Rodenticide Regulation Text

DELIBERATIVE DRAFT

Article 5 - Use Requirements (§ 6453 to 6489)

§ 6471 - Brodifacoum, Bromadiolone, Chlorophacinone Difenacoum, ~~and~~ Difethialone, Diphacinone, Diphacinone sodium salt, Warfarin and Warfarin sodium salt

This section supplements the label restrictions on the use of brodifacoum, bromadiolone, chlorophacinone difenacoum, ~~and~~ difethialone, diphacinone, diphacinone sodium salt, warfarin and warfarin sodium salt. For the purposes of this section, these active ingredients will collectively be referred to as anticoagulant rodenticides.

(a) ~~It is prohibited to place any above ground bait more than 50 feet from a man-made structure unless there is a feature associated with the site that is harboring or attracting the pests targeted on the label between the 50-foot limit and the placement limit specified on the label.~~
Except as provided in (d), use in and around man-made structures is only allowed at:
(1) Health facilities, as defined in California Health and Safety Code (HSC) § 1250
(2) Clinics, as defined in HSC § 1200
(3) Outpatient settings, as defined in HSC § 1248
(4) Locations storing, collecting, or distributing biologics (as defined in HSC § 1600.1) or human tissue or organs (as defined in HSC § 1635)
(5) Pharmacies, as defined in BPC 4037
(6) FDA-registered and inspected facilities involved in commercial manufacture, preparation, compounding, of drugs
(7) Grocery stores, as defined in HSC § 113948
(8) Permanent food facilities, as defined in HSC § 113849
(9) Food processing facilities, as defined in HSC § 109947
(10) Locations with the primary purpose of producing, storing, holding, or packing an agricultural commodity, livestock, poultry, or fish.

(b) Except as provided in (d), it is prohibited to place any above ground bait more than 50 feet from a listed man-made structure, unless there is a feature associated with the site that is harboring or attracting the pests targeted on the label between the 50-foot limit and the placement limit specified on the label.

(c) Except as provided in (d), applications must not exceed 35 consecutive days. All unconsumed bait must be collected at the end of the 35-day period. Double bag and dispose of bait according to the pesticide label directions. The combined application duration of anticoagulant rodenticides at a site must not exceed a total sum of 105 days within a calendar year.

(d) Use is allowed, and exempt from the restrictions in (a), (b), and (c):

(1) For the eradication of nonnative invasive species inhabiting or found to be present on offshore islands in a manner that is consistent with all otherwise applicable federal and state laws and regulations.

(2) If the Department of Fish and Wildlife determines use is required to control or eradicate an invasive rodent population for the protection of threatened or endangered species or their habitats.

(3) To control an actual or potential rodent infestation associated with a public health need, as determined by a supporting declaration from the State Public Health Officer or a local public health officer. For purposes of this section, a public health need is an urgent, nonroutine situation posing a significant risk to human health in which it is documented that other rodent control alternatives, including nonchemical alternatives, are inadequate to control the rodent infestation.

(4) When used by an employee or contractor of a governmental agency or public utility, as defined in Section 216 of the Public Utilities Code, for purposes of protecting water supply and hydroelectric energy generating infrastructure and facilities in a manner that is consistent with all otherwise applicable federal and state laws and regulations.

(5) When used by a governmental agency employee who complies with Section 106925 of the Health and Safety Code to protect public health or by a mosquito abatement and vector control district formed under Chapter 1 (commencing with Section 2000) of Division 3 or Chapter 8 (commencing with Section 2800) of Division 3 of the Health and Safety Code to protect public health.

(6) When FGARs are used at a location with the primary purpose of producing, storing, holding, or packing an agricultural commodity, livestock, poultry, or fish.

(7) For research purposes. Before using a department-registered anticoagulant, a written authorization for research shall be obtained from the director. The director may specify the conditions in the authorization for research under which the research shall be conducted. The director may terminate, amend, or refuse to issue an authorization for research if the director determines any of the following:

(A) The research may involve a hazard to the environment.

(B) The research may be used for purposes unrelated to pesticide data development.

(C) A violation of the authorization for research, prior authorization for research, or Division 6 (commencing with Section 11401) or this division, or a regulation adopted pursuant to either or both of those divisions, has occurred in connection with the research.

§ 6471.5 Sustainable Rodent Management training and plan

For all uses of anticoagulant rodenticides, subsections (a) and (b) apply:

(a) Sustainable Rodent Management Training Course. Commencing one year from the effective date of the regulations, a sustainable rodent management course approved by the Director must be completed each calendar year by every person applying or supervising the application of anticoagulant rodenticides. The course must include Integrated Pest Management and Sustainable Pest Management principles as defined in sections 11401.7 and 11412 of the Food and Agricultural Code respectively, including at a minimum:

(A) Anticoagulant rodenticide non-target effects,
(B) Rodent biology, zoonotic diseases, and identifying target rodents,
(C) Inspection & monitoring,
(F) Sanitation & exclusion,
(E) Anti-rodent landscaping,
(F) Pest management thresholds,
(G) Non-chemical rodent management options,
(H) Rodent management methods & toxicity scales,
(I) Resistance prevention & product rotation,
(J) Safe carcass handling & disposal,
(K) Safe rodenticide storage & disposal site information,
(L) Anticoagulant rodenticides use requirements (CCR Article 5)
(M) Maintaining records

(1) The employer and certified private or commercial applicator as defined in section 6000 must maintain a written record of training course attendance for two years following the date of completion at a central location at the workplace accessible to employees and be provided to the employee, Director, or commissioner upon request. The record must include:

(A) Applicator or handler's name;
(B) License or certificate number if applicable;
(C) Title of the course;
(D) Name of the course provider;
(F) Course completion date;
(G) The applicator or handler's signature confirming attendance.
Other records of course attendance, such as the records required by section 6513, can be used to fulfill this requirement.

DPR Draft Proposed Anticoagulant Rodenticide Regulation Text

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(b) Sustainable Rodent Management Plan. Commencing one year from the effective date of the regulations, before using anticoagulant rodenticides, each business location, certified commercial applicator, or operator of the property must have a written general Sustainable Rodent Management Plan and maintain records. This plan can be general (i.e., not required to be site-specific) and must be reviewed each calendar year and updated as necessary.

(A) In instances where anticoagulant rodenticides are not exclusively applied by pest control businesses, the operator of the property is required to develop a general Sustainable Rodent Management Plan and maintain records.

(B) The operator of the property must provide a copy of their general Sustainable Rodent Management Plan and records to any hired business applying anticoagulant rodenticides on their property.

(1) The written general Sustainable Rodent Management Plan must reflect Integrated Pest Management and Sustainable Pest Management as defined in FAC section 11401.7 and section 11412 respectively and must include the following elements at minimum:

(A) Identifying target rodents,
(B) Inspection & monitoring,
(C) Sanitation & exclusion,
(D) Anti-rodent landscaping,
(E) Pest management thresholds,
(F) Non-chemical rodent management options,
(G) Rodent management methods & toxicity scales,
(H) Resistance prevention & product rotation,
(I) Safe carcass handling & disposal,
(J) Safe rodenticide storage & disposal site information,
(K) Maintaining records.

(2) The pest control business, certified commercial applicator or the operator of the property shall maintain records for all locations where anticoagulant rodenticides are applied. These records must list applicator name, location address, dates anticoagulant rodenticides were deployed and collected, number of anticoagulant rodenticide bait boxes deployed, and U.S. Environmental Protection Agency Registration Number and brand name of anticoagulant rodenticide products used. Records shall be maintained at a central location for two years.

(3) The current and prior written general Sustainable Rodent Management Plan must be available for inspection by the Director or commissioner upon request. Prior copies of the plan must be retained for two years.

(4) Pest control businesses and applicators using anticoagulant rodenticides must follow relevant components of the General Rodent Management Plan when making decisions to apply anticoagulant rodenticides.

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Article

Pre- and Postnatal Exposures to Residential Pesticides and Survival of Childhood Acute Lymphoblastic Leukemia

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Simple Summary: Pesticides have been linked to an increased risk of developing childhood leukemia, yet their impact on survival remains unclear. This study examines whether reported use of pesticides at home before and after birth influences five-year survival in children with acute lymphoblastic leukemia. Our data showed that exposure to pesticides during pregnancy, particularly rodenticides, was linked to a higher risk of death after accounting for other prognostic factors. These findings highlight the need to examine preventable environmental factors that may affect childhood leukemia outcomes, with the goal of improving survival.

Abstract: Background: Exposure to pesticides has been associated with an increased risk of developing childhood leukemia. However, the impact of pesticides on childhood leukemia survival has not been examined. We investigated the associations between residential pesticide use during key developmental periods and 5-year survival in children treated for acute lymphoblastic leukemia (ALL). **Methods:** Residential use of insecticides, herbicides, rodenticides, and flea control products from preconception up to 12 months prior to diagnosis and sociodemographic characteristics were collected via parental interview among 837 children diagnosed with ALL between 1995 and 2008 in California, USA. Data on clinical features were abstracted from medical records. Vital status was obtained through linkage to the National Death Index (NDI) up to 2020. Cox proportional hazards regression models were used to estimate hazard ratios (HRs), adjusting for sociodemographic factors and clinical risk group. **Results:** A total of 108 children with ALL (~13%) died within 5 years of diagnosis. Exposure to any pesticides pre- and/or postnatally was slightly higher among deceased compared to alive children (95.4% vs. 91.5%; $p = 0.23$), while use of rodenticides was significantly higher in children who died (25.0%) vs. those who survived (15.5%; $p = 0.02$). In fully adjusted models, exposure to rodenticides was associated with an increased risk of mortality (HR 1.70; 95% confidence interval (CI) 1.08–2.64; $p = 0.02$), especially when the child was exposed during pregnancy (HR 1.90; 95% CI 1.15–3.16; $p = 0.01$) and possibly 12 months before diagnosis (HR 1.60; 95% CI 0.98–2.61; $p = 0.06$). Increased hazards of death were also observed with other types of pesticides during pregnancy, but those associations were not statistically significant. **Conclusions:** This study is the first to report reduced survival among children with ALL previously exposed to rodenticides, particularly during pregnancy, underscoring the need to further evaluate

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mechanisms by which environmental exposures during key developmental stages may later impact cancer outcomes.

Keywords: childhood acute lymphoblastic leukemia; survival; residential pesticides; rodenticides; developmental periods; pregnancy

1. Introduction

In industrialized countries, leukemia stands as the most prevalent malignancy in children, representing 25% of all pediatric cancers. Among its various subtypes, acute lymphoblastic leukemia (ALL) is the dominant contributor, encompassing 78% of all childhood leukemia cases [1]. Despite its relative prevalence, leukemia in children remains a rare disease, with an incidence of 39 cases per million, peaking between the ages of 2 and 5, and exhibiting heightened rates among the Latinx population in the United States [1–3]. Childhood leukemia originates from genetic disruptions, often beginning in utero with oncogenic fusion proteins, followed by additional hits postnatally [2].

Pesticides—used to control unwanted plants, insects, and animals—expose children through ingestion, inhalation, and skin contact from home use, agricultural drift, and contaminated food. Chronic low-level pesticide exposure, particularly from residential and occupational sources during pregnancy, is linked to higher childhood leukemia risk [4–10].

Environmental chemicals may also contribute to cancer initiation and progression via DNA damage, oxidative stress, and immune reactions [2,11,12]. A metanalysis showed that children from lower socioeconomic backgrounds experience a survival gap compared to those from higher socioeconomic backgrounds [13]. Racial disparities also persist, with higher mortality rates among Latinx and Black patients, attributed to factors like genetics, language barriers, and treatment responses [14,15].

Prognostic factors such as socioeconomic status (SES), race, and ethnicity are interlinked with environmental exposures [16], but little is known about the potential independent impact of chemicals on cancer survival. Few studies have examined the link between environmental exposures during perinatal development and pediatric leukemia survival. A Spanish study found maternal smoking during pregnancy and postnatally increased mortality 4-fold, adverse events 8-fold, and treatment-related mortality 14-fold [17]. Data from the California Childhood Leukemia Study (CCLS) showed that paternal preconception smoking and passive smoke exposure reduced 5-year overall survival [18]. Poor perceived air quality and fine particulate matter levels were also associated with lower survival in childhood ALL, lymphomas, and other cancers [19–22]. The impact of pesticides has been investigated for cancer survival in adults but not children [23,24].

This study leverages data from the CCLS, a case–control study designed to investigate environmental and genetic risk factors for childhood leukemia [18]. We aim to evaluate whether pre- and postnatal residential pesticide exposures influence survival outcomes in children diagnosed with ALL in California.

2. Materials and Methods

The CCLS is a case–control study that includes incident cases of childhood leukemia from hospitals across California and matched population-based controls; cases enrolled from 1995 to 2008 were evaluated in this study. Patients with childhood leukemia were enrolled around the time of diagnosis at 17 hospitals if they were younger than 14 years old at diagnosis, had an English or Spanish-speaking parent, lived in one of the study counties at diagnosis, and had no previous cancer. Interviews with a parent, primar-

ily mothers, used a structured questionnaire to collect information on residential use of 12 types of pesticides (yes/no) during three critical developmental periods, including three months before conception, pregnancy, and postnatally (until the child turned three or was diagnosed, whatever occurred first), as well as within a year of the interview following the leukemia diagnosis. The process of extracting sociodemographic data, leukemia type, and vital status through medical record abstraction, clinician validation, and probabilistic linkage to electronic death certificate data was described in a previous study [18]. Of the 837 consenting ALL cases with completed interviews, 108 were linked to death records, with 5 deaths due to external causes.

The primary exposure of interest was pesticide use. Based on their intended pest targets, the 12 pesticide types were grouped into four broad categories: insecticides for controlling various household and lawn insects (5 types), herbicides for targeting various unwanted plants (2 types), flea control for managing fleas on pets and in living areas (4 types), and rodenticides for controlling rodents (1 type). The total number of pesticide types were also categorized into three exposure levels—low (0–2 types), medium (3–4 types), and high (5–12 types)—based on the tertile distribution.

The outcome evaluated was 5-year survival from all causes except external causes. The nonparametric Kaplan–Meier estimator was used to estimate the survival function and survival curves by pesticide exposure group. A directed acyclic graph (DAG) (Figure S1) identified covariates and their relationships with exposure and outcome. Backward elimination further refined the model, excluding birth weight and household dependents due to minimal impact on risk estimates (<10%). The goodness-of-fit tests using Bayesian Information Criterion (BIC) values for the models with and without these covariates are provided in the Supplemental Materials (Table S1). Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs), adjusting for birth year, parental highest education attained (dichotomized—high school or lower vs. some college or more), annual household income (six categories), race and ethnicity (five categories), and National Cancer Institute (NCI) risk group for ALL (categorical: "standard", defined as age > 1 year and age < 10 years and WBC < 50,000/μL; "high", defined as age $\geq$ 10 years or age > 1 year and age < 10 years and WBC $\geq$ 50,000/μL; and "infant", defined as age < 1 year) [25]. Adjusted Cox models were run with and without SES variables to assess their potential impact on survival outcomes.

Stratified analyses by breastfeeding duration and race and ethnicity were conducted to explore potential effect modification. Breastfeeding duration was considered due to its known influence on early immune development [26], while race and ethnicity were analyzed to account for potential sociodemographic disparities in health outcomes [15,27]. Heterogeneity in HRs for pesticide groups across critical developmental periods was tested using log-rank tests. Log-likelihood ratio tests were used to assess the goodness-of-fit and effect modification by breastfeeding and race and ethnicity. Analyses were performed in the R environment, version 4.3.1 (16 June 2023) [28]. All tests were two-sided, and p-values < 0.05 were considered statistically significant.

This study was approved by the Institutional Review Boards at the University of California, Berkeley, and the California Department of Public Health.

3. Results

3.1. Population Characteristics

Among 837 children with ALL, 47% were Latinx, 35% were non-Latinx Whites, and the remainder were either Asian/Pacific Islander, Black, or of unknown origin. A total of 131 (16%) children came from households with an income below $15,000, and 129 (15%) children lived in households with more than six dependents. Additionally, 36% of parents

had a high school education or less. As presented in Table 1, children who died were more likely to have high-risk ALL, to be diagnosed before the age of one year, and belong to families with low educational attainment and low annual income. Racial disparities were evident, with non-Latinx Black children exhibiting the highest percentage of deceased, followed by Latinx children. There was a suggestion that children who were not breastfed were more likely to die compared to those who were breastfed. The distributions of sex (assigned at birth), number of dependents in the household, birthweight, and gestational age were similar between children who survived and those who did not. Overall, Latinx households and those with low annual income and low education attainment were less likely to use pesticides (Table S2).

Table 1. Characteristics of 837 children with acute lymphoblastic leukemia both overall and stratified by 5-year survival status at the end of 2020—the California Childhood Leukemia Study.

Characteristics	Overall *n* = 837	Alive *n* = 729	Deceased *n* = 108
	n (%)	*n* (%)	*n* (%)
Sex (assigned at birth)			
Female	366 (43.7)	324 (44.4)	42 (38.9)
Male	471 (56.3)	405 (55.6)	66 (61.1)
Race and Ethnicity			
Latinx	396 (47.3)	340 (46.6)	56 (51.9)
Non-Latinx White	295 (35.2)	272 (37.3)	23 (21.3)
Non-Latinx Asian/Pacific Islander	73 (8.7)	61 (8.4)	12 (11.1)
Non-Latinx Black	24 (2.9)	16 (2.2)	8 (7.4)
Other/Unknown	49 (5.9)		
Birth Years			
1982–1989	64 (7.6)	48 (6.6)	16 (14.8)
1990–1999	509 (60.8)	442 (60.6)	67 (62.1)
2000–2014	264 (31.5)	239 (32.8)	25 (23.1)
Household Annual Income (USD)			
<15,000	131 (15.7)	112 (15.4)	19 (17.6)
15,000–29,999	149 (17.8)	123 (16.9)	26 (24.1)
30,000–44,999	130 (15.5)	112 (15.4)	18 (16.7)
45,000–59,999	122 (14.6)	102 (14.0)	20 (18.5)
60,000–74,999	63 (7.5)	57 (7.8)	6 (5.5)
75,000+	242 (28.9)	223 (30.6)	19 (17.6)
Number of Dependents in the Household			
1–3	184 (22.0)	155 (21.2)	29 (26.9)
4–5	524 (62.6)	459 (63.0)	65 (60.1)
6+	129 (15.4)	115 (15.8)	14 (13.0)
Highest Parental Education Attained			
High School or Lower	303 (36.2)	260 (35.7)	43 (39.8)
Some College or More	533 (63.7)	468 (64.2)	65 (60.2)
Unknown	1 (0.1)		
Age at Diagnosis (years)			
<1	27 (3.2)	13 (1.8)	14 (13.0)
1–2	195 (23.3)	175 (24.0)	20 (18.5)
3–6	391 (46.7)	360 (49.4)	31 (28.7)
7–9	103 (12.3)	86 (11.8)	17 (15.7)
10–14	121 (14.5)	95 (13.0)	26 (24.1)

Table 1. *Cont.*

Characteristics	Overall n = 837	Alive n = 729	Deceased n = 108
NCI Risk Group			
Standard	561 (67.0)	509 (69.8)	52 (48.1)
High	226 (27.0)	186 (25.5)	40 (37.0)
Infant	26 (3.1)	12 (1.7)	14 (13.0)
Unknown	24 (2.9)		
Birthweight (grams)			
<2500	39 (4.7)	34 (4.7)	5 (4.6)
2500–4000	663 (79.2)	580 (79.6)	83 (76.9)
>4000	135 (16.1)	115 (15.8)	20 (18.5)
Gestational Age (weeks)			
<36	45 (5.4)	36 (4.9)	9 (8.3)
36–41	608 (72.6)	535 (73.4)	73 (67.6)
41+	175 (20.9)	149 (20.4)	26 (24.1)
Unknown	9 (1.1)		
Breastfeeding			
No	138 (16.5)	114 (15.6)	24 (22.2)
Yes	663 (79.2)	585 (80.2)	78 (72.2)
Unknown	36 (4.3)		
Breastfeeding Duration (months)			
6 or Less	520 (62.1)	450 (61.7)	70 (64.8)
More than 6	281 (33.6)	249 (34.2)	32 (29.6)
Unknown	36 (4.3)		

Percentages may not amount to 100% due to rounding. Abbreviations: USD—United States Dollar; NCI—National Cancer Institute.

3.2. Bivariate Analyses

About 92% of all children with ALL were exposed to at least one pesticide type pre- and/or postnatally. The use of pesticides tended to be correlated across different periods. Rodenticide exposure, for instance, showed strong correlations between preconception and pregnancy (correlation coefficient r = 0.82) and between pregnancy and postnatal periods (r = 0.78), suggesting consistent use over time (Figure S2). Conversely, correlations between different pesticide categories were generally low, indicating distinct patterns of use.

Bivariate analysis showed no significant difference in survival between children ever exposed to pesticides and those never exposed (p = 0.23, Table 2). However, survival was lower in children exposed to rodenticides, with 25% exposed among the deceased compared to 15.5% among survivors (p = 0.02). Exposure to rodenticides pre- and postnatally was associated with lower survival rates (80–83%) compared to unexposed children (85–90%) or those exposed to other pesticides (Table S3). The Kaplan–Meier curves showed a statistically significant decrease in 5-year survival in the group exposed to rodenticides at any time (p = 0.015; Figure 1) and during pregnancy (p = 0.022; Figure 2).

Table 2. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models without and with adjustments for socioeconomic status.

Exposure	Alive *n* = 729	Deceased *n* = 108		Model 1—Without SES Adjustment *		Model 2—With SES Adjustment **	
	n (%)	*n* (%)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Any Pesticides							
No	62 (8.5)	5 (4.6)		Ref.		Ref.	
Yes	667 (91.5)	103 (95.4)	0.23	2.06 (0.83–5.11)	0.1	2.22 (0.89–5.54)	0.09
Number of Types Used							
0–2 (Low)	327 (44.9)	40 (37.0)		Ref.		Ref.	
3–4 (Medium)	226 (31.0)	42 (38.9)		1.67 (1.07–2.59)	0.02	1.77 (1.14–2.77)	0.01
5–12 (High)	176 (24.1)	26 (24.1)	0.17	1.47 (0.88–2.44)	0.14	1.56 (0.93–2.62)	0.09
Insecticides							
No	110 (15.0)	15 (13.9)		Ref.		Ref.	
Yes	619 (84.9)	93 (86.1)	0.86	1.10 (0.63–1.91)	0.7	1.15 (0.66–2.00)	0.6
Herbicides							
No	362 (49.7)	54 (50.0)		Ref.		Ref.	
Yes	367 (50.3)	54 (50.0)	1	1.16 (0.78–1.72)	0.5	1.31 (0.87–1.98)	0.2
Flea Control							
No	419 (57.5)	64 (59.3)		Ref.		Ref.	
Yes	310 (42.5)	44 (40.7)	0.8	1.16 (0.72–1.57)	0.8	1.04 (0.70–1.54)	0.8
Rodenticides							
No	614 (84.2)	81 (75.0)		Ref.		Ref.	
Yes	113 (15.5)	27 (25.0)	0.02	1.75 (1.13–2.72)	0.01	1.69 (1.08–2.64)	0.02
Unknown (*n* = 2)							

Percentages may not amount to 100% due to rounding. Abbreviations: HR—hazards ratio; CI—confidence interval; Ref—reference; SES—socioeconomic status. * Adjusted for age at diagnosis, race and ethnicity, and NCI risk group status. ** Adjusted for age at diagnosis, race and ethnicity, NCI risk group status, highest parental education attained, and household income.

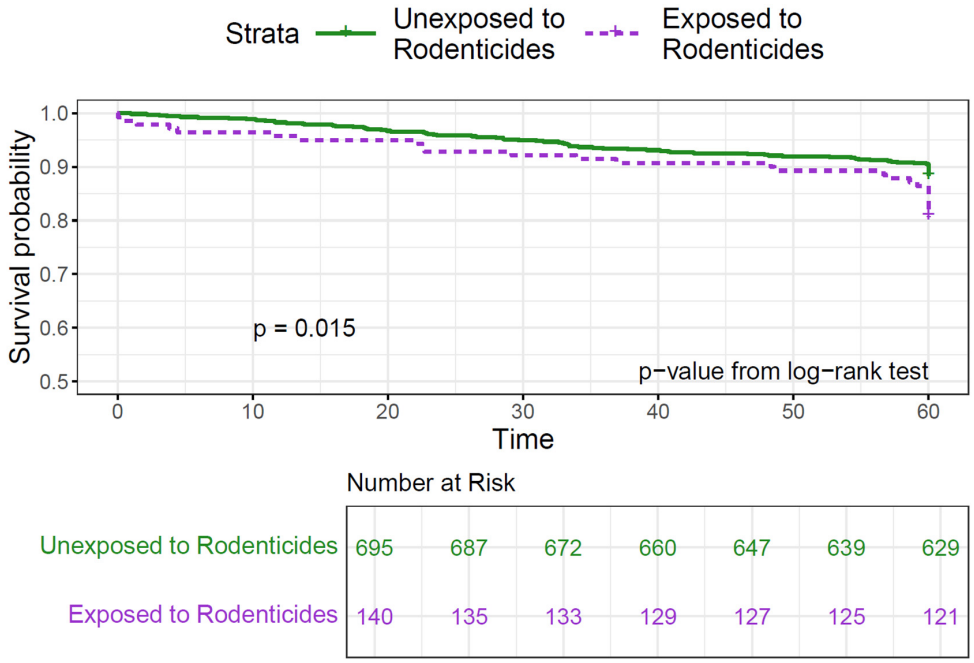

Figure 1. Kaplan–Meier curves for 5-year survival in childhood ALL by rodenticide exposure during any time period.

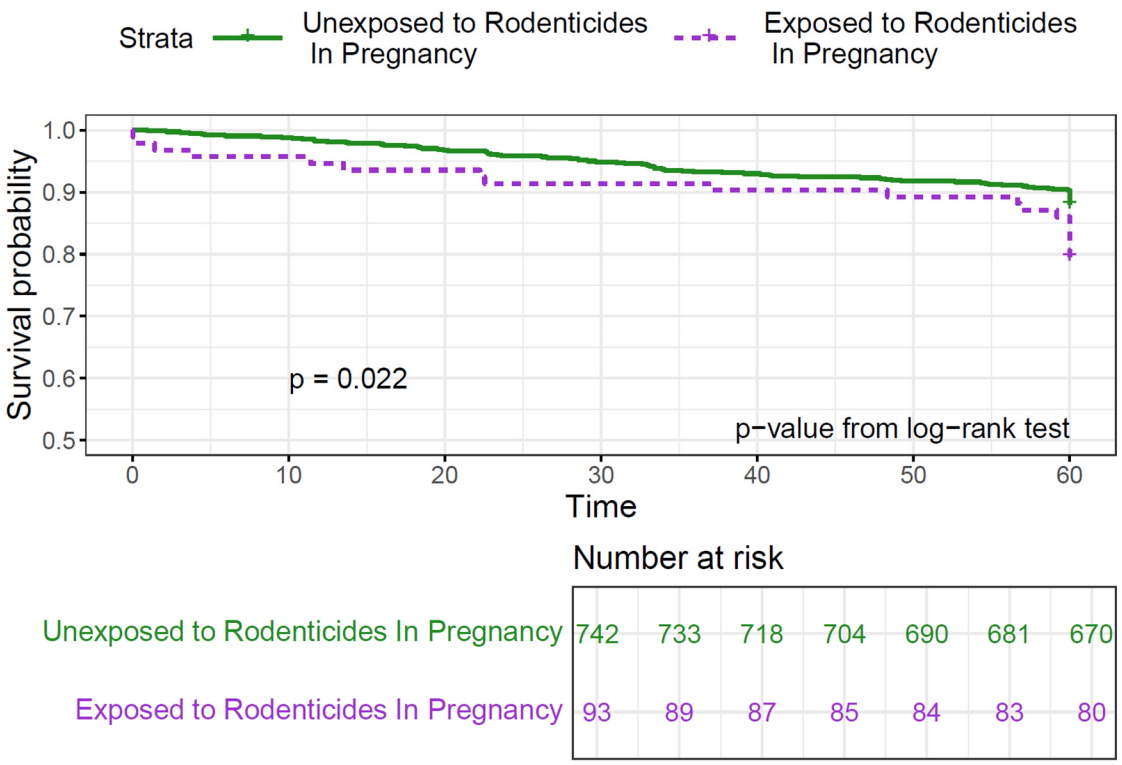

Figure 2. Kaplan–Meier curves for 5-year survival in childhood ALL by rodenticide exposure during pregnancy.

3.3. Multivariate Analyses

Table 2 shows the HRs for 5-year survival of childhood ALL in relation to pesticide exposure at any time, with adjustment for age at diagnosis, race and ethnicity, and NCI risk group (Model 1) and additional adjustment for SES, including parental education and annual household income (Model 2). A two-fold increased risk of mortality was associated with overall exposure to any pesticides in both models but did not reach statistical significance (p = 0.09). Increased risk of mortality was associated with exposure to rodenticides in Model 1 (HR 1.75; 95% CI: 1.13–2.72) and Model 2 (HR 1.69; 95% CI: 1.08–2.64). There was no clear dose–response relationship when examining the number of pesticide types used.

In fully adjusted Cox proportional hazards models examining childhood ALL survival by pesticide exposure during various developmental periods (Table 3), statistically significant increased risks of mortality were seen with exposure to any pesticide during pregnancy (HR 1.6; 95% CI 1.05–2.42), mostly driven by rodenticides (HR 1.91; 95% CI 1.15–3.16) and possibly insecticides and herbicides that conferred 45 to 50% increased risks of mortality, although falling short of statistical significance. Additionally, data suggested that children exposed to rodenticides 12 months prior to the interview had a 60% increased risk of mortality (p = 0.06). No significant differences were observed for other pesticide categories across different developmental periods. Adjusting for highly correlated variables challenged model robustness. Sensitivity analysis adjusting across different time windows yielded consistent trends with the primary findings on rodenticide exposure during pregnancy compared to those unexposed (HR 3.12; 95% CI 1.01–9.65) (Table S4). Similarly, rodenticide exposure during pregnancy yielded an HR of 1.74 (95% CI 1.02–2.99) compared to those who were unexposed, after adjusting for other pesticide use during pregnancy (Table S5).

Table 3. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by periods of exposure.

Exposures	Preconception		Pregnancy		Postnatally		12 Months Before Interview	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Any Pesticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.02 (0.68–1.52)	>0.90	1.60 (1.05–2.42)	0.03	0.84 (0.53–1.35)	0.5	1.35 (0.87–2.09)	0.2
Insecticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.00 (0.67–1.48)	>0.90	1.45 (0.96–2.17)	0.08	0.81 (0.53–1.25)	0.3	1.13 (0.75–1.71)	0.6
Herbicides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.22 (0.79–1.88)	0.4	1.50 (0.98–2.29)	0.06	1.10 (0.73–1.65)	0.7	1.26 (0.84–1.90)	0.3
Flea Control								
No	Ref.		Ref.		Ref.		Ref.	
Yes	0.90 (0.57–1.43)	0.7	1.05 (0.68–1.64)	0.8	0.83 (0.55–1.26)	0.4	1.11 (0.74–1.68)	0.6
Rodenticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.49 (0.85–2.63)	0.2	1.91 (1.15–3.16)	0.01	1.46 (0.91–2.33)	0.1	1.60 (0.98–2.61)	0.06

Abbreviations: HR—hazards ratio; CI—confidence interval; Ref—reference. * Adjusted for age at diagnosis, race and ethnicity, highest parental education attained, household income, and NCI risk group status.

3.4. Effect Modification

Stratified analyses by racial and ethnic group showed that rodenticide exposure was associated with poorer survival among non-Latinx White children (HR 3.35; 95% CI 1.42–7.88), while a weaker association was observed in Latinx children (HR 1.66; 95% CI 0.91–3.01) (Table 4). No significant associations were seen in other racial/ethnic groups. Formal testing for effect modification confirmed that survival varied by race and ethnicity (p for interaction = 0.02).

Table 4. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by race and ethnicity.

Exposure	Non-Latinx White		Latinx		Non-Latinx Black + Asian + Others		Interaction *p*-Value
	HR (95%CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	
Insecticides	1.02 (0.24–4.41) 265/21	>0.90	1.41 (0.69–2.9) 317/47	0.3	0.70 (0.22–2.26) 130/25	0.6	0.8
Herbicides	1.00 (0.41–2.45) 197/15	0.9	0.54 (0.88–2.68) 141/23	0.1	1.40 (0.59–3.28) 83/16	0.4	0.8

Table 4. *Cont.*

Exposure	Non-Latinx White		Latinx		Non-Latinx Black + Asian + Others		Interaction *p*-Value
	HR (95%CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	
Flea Control	0.83 (0.36–1.90) 164/12	0.7	1.55 (0.91–2.64) 145/26	0.1	0.55 (0.22–1.39) 45/6	0.2	0.09
Rodenticides	3.35 (1.42–7.88) 46/9	0.005	1.66 (0.91–3.01) 73/16	0.1	0.45 (0.10–1.93) 21/2	0.3	0.02

Abbreviations: HR—hazards ratio; CI—confidence interval. * Adjusted for age at diagnosis, race and ethnicity, highest parental education attained, household income, and NCI risk group status.

Stratified analyses by breastfeeding duration suggested that children exposed to insecticides and who were not breastfed or breastfed for 6 months or less had a higher risk of dying (HR 1.83; 95% CI 0.82–4.08) compared to those unexposed. In contrast, among children breastfed for more than 6 months, insecticide exposure was not associated with increased mortality risk (HR 0.82; 95% CI 0.31–2.12). Formal tests for effect modification, however, did not reach statistical significance (p for interaction = 0.11) (Table S6).

4. Discussion

4.1. Key Findings

Our study, based on comprehensive interview data from Californian families, suggests a significant association between exposure to any residential pesticides during pregnancy and lower survival in children with ALL, after adjusting for clinical and sociodemographic factors. This association was mostly driven by exposure to rodenticides, and to a lesser extent insecticides and herbicides. These findings emphasize the vulnerability of pesticide-exposed patients, highlighting the impact of exposure prior to diagnosis.

To our knowledge, no other childhood cancer study has investigated the relationship between pesticide exposure and survival. Data among adults are also scarce. A 2019 French study examined lymphoma patients with occupational pesticide exposure and found reduced response to immunochemotherapy and lower survival [23]. Another study of Hodgkin's lymphoma patients residing near agricultural fields in California observed no significant association between environmental pesticide exposure and survival [24]. The lack of convergence among adult studies may be due to differences in routes and levels of exposure to agricultural pesticides.

We examined pesticide exposures during key developmental periods and their effects on leukemia survival rates in children. The pregnancy period was particularly impactful, as mortality was associated with exposure to both any pesticide category and rodenticides. Sensitivity analyses adjusting for collinearity indicated that rodenticide exposure during pregnancy significantly increased the hazard of mortality, highlighting pregnancy as a critical period. Other pesticides showed no significant associations with mortality across different time windows.

In general, the results from Models 1 and 2 (Table 2) were similar, showing little impact from social factors. To account for the additional confounding effects of healthcare access and financial burden on treatment outcomes, we ran sensitivity analyses adjusting for hospital site and number of household dependents, which did not substantially alter the observed association between rodenticide exposure and poor survival.

Latinx households and those with lower income and education levels reported using fewer pesticides than other groups (Table S2), which may reflect differences in household practices, access to pesticide products, or awareness of risks associated with pests. Stratified

analyses indicated differences in the association between pesticide exposure and survival, particularly for rodenticide exposure among non-Latinx White children, possibly due to the disproportionately large number of exposed individuals in this group (Table S1). Smaller sample sizes for racial and ethnic groups limit the robustness of these findings, emphasizing the need to further examine socioeconomic disparities and conduct larger-scale studies [13,16,27,29]. In addition, interview data are somewhat limited in characterizing the levels of exposure to pesticides with precision, therefore limiting the interpretation of our results showing no overall dose–response relationship with survival.

Building on evidence linking short breastfeeding duration to childhood leukemia risk [26,30–33], our data suggest higher pesticide-related survival risks in children breastfed for less than six months. Though interaction tests were not significant, this warrants further study on immune modulators' roles in cancer relapse and survival, particularly in mitigating the adverse survival effects associated with rodenticide exposure.

4.2. Biological Effects of Pesticides

Population studies have consistently linked residential pesticide exposure to an elevated risk of developing childhood leukemia, emphasizing both in utero and postnatal exposures [6,34–36]. Like other leukemogenic agents, such as etoposide, benzene metabolites, and lack of bioflavonoids, certain pesticides exert toxicity through oxidative stress and mitochondrial dysfunction. These processes can induce DNA breaks, potentially leading to chromosomal rearrangements (duplications, deletions, and translocations) if not properly repaired [37]. The initial impact often occurs in utero, giving rise to oncogenic fusion proteins. Subsequent insults, determining disease latency, occur post-birth and may involve genetic, epigenetic, or immune factors (e.g., delayed infection-mediated immune deregulation) [2]. Studies suggest that pesticides like organophosphates, carbamates, and pyrethroids—commonly present in insecticides and herbicides—can impair leukocyte function by inducing apoptosis, arresting the cell cycle, and disrupting immune cell functions [38]. Distinct patterns in chromosomal aberrations, cytologic features, and peripheral blood and bone marrow indices (similar to those found in patients with secondary leukemia typically induced by radiation or chemotherapy) have been documented in adult patients with acute myeloblastic leukemia who have been exposed to pesticides (n = 21) vs. those not exposed (n = 40) [39]. The authors suggested that pesticide exposure may worsen leukemia prognosis and survival by triggering harder-to-treat cytogenetic and clinical subtypes. Overall, epidemiological and biological data support the role of certain pesticides in both the development and prognosis of leukemias.

4.3. Rodenticides: Prevalence and Potential Health Risks

Household and agricultural rodenticide use is common, resulting in over 8000 calls to poison centers in 2021 [40]. Most commonly, these calls are related to ingestion, either intentional or unintentional. It is uncertain how often or to what degree incidental small exposures not prompting calls occur. Rodenticides are used in bait stations and have a low risk of volatilization [41,42] that minimizes the likelihood of exposures of acute significance, but undocumented low-dose exposures may occur relatively commonly in the process of opening, moving, or disposing of bait stations. We could not identify any biomonitoring studies examining this kind of low-dose exposure. In addition, there have been many reported cases of illegally imported chemicals used as rodenticides, such as tetramethylenedisulfotetramine and aldicarb, with entirely different mechanisms of action, causing acute illness in the U.S. [43,44]. The degree to which these factors support the biologic plausibility of our findings is uncertain.

Rodenticides include non-anticoagulants like bromethalin, a neurotoxic compound that disrupts oxidative phosphorylation, leading to cytotoxic edema, though human exposure reports suggest it is found in sub-lethal concentrations with no clear dose–response threshold [45,46]. They also contain anticoagulants like brodifacoum, a potent second-generation "super warfarin" that inhibits vitamin K recycling and disrupts blood clotting [47]. It was found in d-Con, the primary rodent-control product used by participants in our study, at a concentration of 0.005% until 2015, when the EPA banned its residential use. However, brodifacoum remains widely used in professional and agricultural settings [48]. Brodifacoum inhibits vitamin K epoxide reductase (VKOR), disrupting the vitamin K cycle, reducing clotting factor synthesis, and prolonging coagulation times [49–51]. Known for its high affinity and prolonged elimination half-life, brodifacoum causes acute poisonings with symptoms resembling fatal leukemia [52]. Our study emphasizes the need to investigate rodenticide exposures' mechanism of effects on leukemia survival, focusing on hematologic and non-hematologic mechanisms tied to vitamin K inhibition [53].

4.4. Limitations and Strengths

Our study leveraged existing data on pesticide exposure during key developmental periods from a case-control study, though reliance on self-reported questionnaires may introduce recall bias influenced by parents' perceptions or societal pressures. However, 73% of ALL cases were diagnosed under the age of 6, likely improving recall accuracy, supported by consistent data across periods, including the reliable 12-month period prior to the interview. High correlation between exposure periods limits our ability to draw definitive conclusions on the relative contributions of prenatal vs. postnatal exposure. While deceased children were not excluded, parents of 50 children who passed away shortly after enrollment did not complete the interview. The survival rate (87%) aligned with national averages (1995–2015) [30,54], and demographic data from the birth registry for these deceased children who did not complete the interview were comparable to the other cases included, supporting representativeness. However, potential differences in neighborhood income between interviewed and non-interviewed families raise concerns about selection bias. Despite adjustments for key sociodemographic factors in our analysis, residual confounding SES factors cannot be ruled out. The causal diagram indicated no need for additional adjustments beyond income, education, and race and ethnicity, and the additional adjustment for hospital sites did not change the results. However, our data did not capture detailed information on access to specialized healthcare, type of medical insurance, or treatment-related factors such as financial resources, drug availability, and access to novel therapies, all of which may influence survival outcomes. Variability in these factors, including timely administration of conventional treatments and management of therapy-related complications, could contribute to disparities in survival [55]. Finally, the limited number of exposed cases, coupled with high correlation of exposure across time periods, constrain the statistical power of our findings and make it challenging to disentangle the independent effect of exposure during a specific time period driving the association.

This study demonstrates key strengths in accounting for potential confounders, largely due to the rich sociodemographic and pesticide exposure data from the CCLS interviews, which provided insights into records of linkage studies, thus enhancing the rigor of our analyses.

5. Conclusions

Our study, featuring detailed data collection and attention to confounders, suggests associations between pesticide exposure—especially for use of rodenticides during pregnancy—and reduced childhood ALL survival. Future studies should aim for more direct exposure assessment methods, larger sample sizes, and a more comprehensive evaluation of leukemia prognosis, including molecular subtypes of ALL and treatment response. By situating our work within the broader context of the impact of environmental exposures on the pediatric cancer continuum from etiology to short- and long-term outcomes, we contribute to the growing body of knowledge on the impact of chemical exposures on childhood leukemia prognosis. This study stands as an initial step towards understanding the effects of pesticide exposure during key developmental stages on the survival outcomes of children with leukemia, urging further research to enhance survival outcomes by addressing preventable environmental factors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers17060978/s1, Table S1: Estimates and goodness-of-fit statistics for model comparison: Cox proportional hazards model for any pesticide exposure among children with acute lymphoblastic leukemia by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S2: Sociodemographic characteristics and pesticide exposure among 837 children with acute lymphoblastic leukemia—the California Childhood Leukemia Study; Table S3: Periods of residential pesticide use by 5-year survival status at the end of 2020 in children with acute lymphoblastic leukemia—the California Childhood Leukemia Study; Table S4. Multivariate analysis of rodenticide exposures adjusted for all time windows among children with acute lymphoblastic leukemia using Cox proportional hazards model by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S5. Multivariate analysis of pesticide exposures in pregnancy period adjusted for other pesticide groups among children acute lymphoblastic leukemia using the Cox proportional hazards model * by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S6. Residential pesticides and 5-year survival at the end of 2020 in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by duration of breastfeeding; Figure S1: Directed acyclic graph (DAG); Figure S2: Correlation matrix between pesticide categories and windows of exposure.

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Institutional Review Board Statement: This study was approved by the Institutional Review Boards at the University of California, Berkeley, and the California Department of Public Health (approval code 2020-167, on 29 August 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the California Childhood Leukemia Study. The Committee for Protection of Human Subjects granted a waiver of consent to also conduct research on outcomes.

Data Availability Statement: The epidemiological and clinical data generated in this study are not publicly available due to the terms of the informed consent signed when subjects were enrolled to the CCLS study but may be available upon reasonable request from the corresponding author. The death data analyzed in this study were obtained from the California Department of Public Health (CDPH) Center for Health Statistics and Informatics (CHSI) and are not publicly available due to terms of CDPH-CHSI.

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Abbreviations

The following abbreviations are used in this manuscript:

ALL	Acute Lymphoblastic Leukemia
BIC	Bayesian Information Criterion
CCLS	California Childhood Leukemia Study
CI	Confidence Interval
DAG	Directed Acyclic Graph
HR	Hazards Ratio
NCI	National Cancer Institute
Ref	Reference
SES	Socioeconomic Status

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Spatial patterns of anticoagulant rodenticides in three species of medium-sized carnivorans in Pennsylvania

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Abstract

Human influences on natural environments are now ubiquitous but manifest in multiple and unique ways depending on local environments and communities. Attempts to control, or mediate, local pests to residences or to agriculture can impart important negative consequences on systems. Secondary exposure to anticoagulant rodenticides (ARs) can cause numerous adverse effects on wild carnivores including death. Few studies have quantified AR prevalence, investigated their pathway of exposure, or associations with specific location types in the northeastern U.S. We hypothesized that ARs would be found in the mesocarnivore community throughout Pennsylvania and have the greatest detection rate in highly urbanized or agricultural landscapes. From 2019 through early 2022, we collected carcasses to obtain liver samples (n = 265) from three species of carnivores: bobcats (*Lynx rufus* (Schreber, 1777)), fishers (*Pekania pennanti* (Erxleben, 1777)), and river otters (*Lontra canadensis* (Schreber, 1777)). We used generalized linear models to test for differences in AR detection rates among species and spatial scales including the six Pennsylvania Game Commission regions and 23 wildlife management units. We detected ARs in all species (44.2% collectively), but detection rates differed among species. Our study is the first to document ARs within North American river otters.

Key words: anticoagulant rodenticides, development, *Lontra canadensis* (Schreber, 1777), *Lynx rufus* (Schreber, 1777), *Pekania pennanti* (Erxleben, 1777), river otter

Introduction

Rodents have been perceived as a threat to human health and livelihood for millennia (Van den Brink et al. 2018). Over the last century, various compounds have been developed to reduce rodent populations, especially those near humans. The most used compounds are anticoagulant rodenticides (ARs), which are further categorized as either first generation (FGAR) or second generation (SGAR) (Rattner et al. 2014), with second-generation compounds developed after resistance to the first generation was identified in some rodents (Jacob and Buckle 2018). In general, second-generation ARs are more potent, longer acting, and more likely to accumulate in tissues than first-generation ARs (Erickson and Urban 2004; Rattner et al. 2014; Elliott et al. 2016), with tissue liver half-lives of up to 350 days (Eason et al. 2002; Fisher et al. 2003; Horak et al. 2018).

Anticoagulant rodenticides inhibit vitamin K epoxide reductase, the enzyme responsible for maintaining adequate vitamin K levels (Watt et al. 2005; Rattner et al. 2014). In 2008, to prevent poisoning of non-target wildlife, children, and domestic animals, the U.S. Environmental Protection Agency (EPA) restricted use of SGARs to agricultural contexts and licensed professionals (e.g., commercial exterminators) (Erickson and Urban 2004; Memmott et al. 2017). Products containing the two most toxic compounds, brodifacoum and difethialone, were still available to the public until 2015 (Murray 2017), and the remaining stockpiles of products containing these two compounds may be available for use for decades to come. Moreover, other second-generation ARs remain readily available (e.g., in hardware or farm supply stores), with widespread exposure to these compounds detected in wildlife around the world. The persistence and toxicity of second-generation ARs render these compounds of particular concern for biomagnification (elevated concentrations) in predators (Horak et al. 2018; López-Perea et al. 2019; Fernandez-de-Simon et al. 2022). Many carnivores readily scavenge carcasses they find on the landscape. Rodents, either recently deceased or suffering rodenticide intoxication, could be encountered, consumed, and provide secondary exposure to carnivores. Notably, Riley and co-workers (Riley et al. 2003) reported acute toxicity from AR exposure as the second leading cause of mortality in coyotes (*Canis latrans* Say, 1823) over a 9-year period in the Santa Monica Mountains National Recreation Area (bordering Los Angeles, California, United States).

The concentrations of ARs that evoke mortality are both poorly understood and apparently highly variable within and among species (Quinn 2019). For example, lethal concentrations from liver samples were found to be as low as 0.17 μg/g for the caracal ((*Caracal caracal* (Schreber, 1776)); Serieys et al. 2019), yet in another wild felid (bobcat (*Lynx rufus* (Schreber, 1777))) individuals with liver concentrations of up to 5.81 μg/g lived for several years (Serieys et al. 2015). A growing area of concern is the sublethal effect of chronic exposure to ARs, which may influence immune function and behavior, potentially affecting an animal's ability to respond to external stimuli (such as predators), thereby putting them at increased risk of mortality beyond the effects of AR intoxication (Serieys et al. 2018*a*, 2018*b*). In laboratory studies, sublethal exposure to ARs produced upwards of 70% mortality when combined with other stressors (Jaques 1959). In a long-term field study of bobcats, secondary AR exposure (at ≥0.05 μg/g wet weight in liver) was associated with severe infestation of notoedric mange (an ectoparasitic disease) (Riley et al. 2007; Serieys et al. 2015). Likewise, a negative association between AR exposure and body condition has been observed in weasels and stoats (Elmeros et al. 2011). Thompson et al. (Thompson et al. 2014) indicated a negative association between AR exposure and fisher survival in California. In studies of humans, dogs, and sheep, the reproductive consequences of AR exposure have included abortions, fetal toxicosis, congenital deformities, and decreased sperm counts (Ginsberg and Hirsh 1989; Robinson et al. 2005; Murray 2017). Although data are limited, Serieys et al. (2015) documented that AR residues transfer from mother to offspring in bobcats. In sum, AR exposure may pose an important challenge for individual fitness and population viability; large-scale analyses evaluating the explicit effects of ARs to populations are difficult to design and execute.

Rodenticide exposure in carnivorous species is widespread where it has been investigated (Riley et al. 2003, 2007; McMillin et al. 2008; Gabriel et al. 2012; Serieys et al. 2015; Rudd et al. 2018; Wiens et al. 2019). The spatial variation in type and extent of exposure, and in the potential for repeated exposure, has been associated with human-dominated landscapes (i.e., commercial, residential, and agricultural areas) because these areas have the highest use of ARs (Cypher et al. 2014). High AR concentrations have been reported in relatively rural and isolated locations when there are activities (e.g., illegal cannabis growing on public lands) where humans deploy large numbers of ARs to control rodents (Gabriel et al. 2012, 2015). Such activities may confound or obfuscate the general patterns associated with AR exposure in wildlife. Nevertheless, on average individual carnivores that live in these areas should have the greatest likelihood of exposure and to any adverse effects of ARs (Hindmarch and Elliott 2018; Serieys et al. 2019). Researchers have shown that some carnivorans like kit foxes (*Vulpes macrotis* Merriam, 1888) may encounter first generation compounds in one habitat type (e.g., undeveloped or agricultural settings) and encounter second-generation compounds in residential or industrial areas (Cypher et al. 2014). Moreover, ARs have been increasingly detected in aquatic or semi-aquatic animals, including some species of freshwater fish, invertebrates, and raptors that forage in aquatic systems (e.g., Bald Eagles (*Haliaeetus leucocephalus* (Linnaeus, 1766))) (Regnery et al. 2020; Niedringhaus et al. 2021). The sources of ARs in these systems are also traceable to urban areas (Regnery et al. 2020).

The northeastern United States supports the highest rural human population densities in the United States, leading to a high degree of human–wildland interface. Eastern forests have relatively high rates of private ownership, which could allow for large and consistent use of ARs with little oversight (L'Roe and Allred 2013). The amount of land cover devoted to agriculture and human populations is large compared to other regions of the United States (Drummond and Loveland 2010; Homer et al. 2020). As a result, carnivorans in the Northeast are likely at high risk of AR exposure. Fishers (*Pekania pennanti* (Erxleben, 1777)) and bobcats are terrestrial predators of conservation and management interest across North America (Powell 1993; Lovallo and Anderson 1996; Powell et al. 2017). Fishers were reintroduced into Pennsylvania (PA) in the late 1990s and have apparently stable or increasing populations across much of the state (Lewis et al. 2012) (Supplementary Fig. S1). Fishers readily scavenge carcasses and are prolific predators of rodents. Bobcats may also scavenge, but are more associated with urban, suburban, and human-modified landscapes than fishers (Lovallo and Anderson 1996; McNeil et al. 2017; Powell et al. 2017). Aquatic and semi-aquatic species have also been identified to have exposure to ARs (Serieys et al. 2019; Niedringhaus et al. 2021); nonetheless, investigations into the exposure of aquatic mammalian species are seldom undertaken. River otters (*Lontra canadensis* (Schreber, 1777)), like fishers, are classified in the family *Mustelidae,* and are semi-aquatic. They, too, will readily scavenge for food in both aquatic and terrestrial environs. Yet river otters primarily prey on fish and aquatic invertebrate species (Liers 1951; Day et al. 2015). Their aquatic habitat and dietary differences provide an important contrast to fishers and bobcats that are indicative of the relative availability of ARs across the landscape. Examining differences in exposure among these three species provides an important test of whether ARs are ubiquitous, confined to specific landcover or habitat types, or a product of the relative life histories and food web of the species.

Here we use fishers, bobcats, and river otters to investigate spatial and inter-specific variation of AR exposure across PA. This set of species represents an important contrast between life histories and major land-use differences that could inform the extent and pathways by which carnivores are being exposed to ARs. These three species are also highly regulated and routinely monitored in PA. Yet, prior to this study, there was no baseline data on AR exposure in these species that could inform or refute the potential roles that ARs may play in their population status or trends. We hypothesized that AR detection would be observable based on differences in habitat use, as indexed by landcover types, and differences in foraging strategy and diets across species. We predicted that fishers would have the highest exposure to ARs followed by bobcats, then river otters. We based this prediction on the observation that fishers occupy terrestrial environments that may be near agricultural or developed areas in Pennsylvania. We expected bobcats to have a reduced exposure because, al-

though they exist in similar habitat as fishers, we hypothesize that they scavenge dead or dying rodents at a reduced rate compared to fishers. Finally, we expected river otters to have the lowest exposure rates because of their use of aquatic food sources, where exposure to ARs would be diminished compared to terrestrial food sources near agricultural or human development. We also evaluated the hypothesis that AR exposure would be highest for all species in areas with the highest total amount of land use dedicated to agriculture or human development (urbanization). Finally, because long-lived animals should have the most opportunity to encounter ARs, we hypothesized that the oldest animals, of all three species, would have the highest rate of exposure.

Materials and methods

Sample collection

We collected carcasses from mesocarnivores from several sources across PA from autumn 2019 through March 2022. Liver samples from 2019 were obtained from carcasses housed in freezers at Pennsylvania Game Commission (PGC) regional offices in PA that had been previously collected as illegal or accidental harvests, nuisance animals, or vehicular collisions. In some instances, these samples had no specific corresponding date or location of collection but were included for analysis to provide a general background estimate of prevalence across the state. Starting in winter of 2020, we worked cooperatively with licensed PA trappers to collect liver samples from recently legally harvested bobcats, fishers, and river otters, and continued to opportunistically collect carcasses and liver samples. The collection of carcasses was coordinated by PGC staff with field support from Wildlife Futures Program personnel. Our design and intent were to gather equal numbers of carcasses from each species in the six PGC regions and to obtain similar numbers from as many wildlife management units ($n = 23$; wildlife management units) as possible. Because samples could only be collected through cooperation with local trappers and regional staff, we could not fully control the numbers or species that came from each wildlife management unit or region of the state. As a result, spatial variation in population numbers (e.g., fishers; Supplementary Fig. S1) and trapping effort led to unequal spatial representation in our data. Both PGC regions and wildlife management units are combinations of ecological, geopolitical, and within-agency administrative units that function to manage wildlife (e.g., allocate license numbers or regulations). Ideally, our study and analyses would focus on the smallest spatial scales that were biologically relevant to the species of research, but wildlife management units are the smallest extent that trappers were required to report their harvested animals. From each carcass, we removed the liver and placed a roughly 100 g portion in a sterile, plastic bag (Whirl-pak™, The Aristotle Corporation, Stamford, CT) that was frozen within 2 h. For each specimen, we recorded the species, sex (if determinable), relative age (juvenile, adult), location of collection to the county or wildlife management units level, and manner of death (e.g., roadkill, legal trapping, etc.). Where possible, we extracted one canine tooth that we submitted to Matson's Laboratory (135 Wooden Shoe Lane, Manhattan, MT 59741, USA) for age estimation (Arthur et al. 1992). For each carcass, we created a unique identifier based on the species, date, and location of collection to match AR data to environmental data.

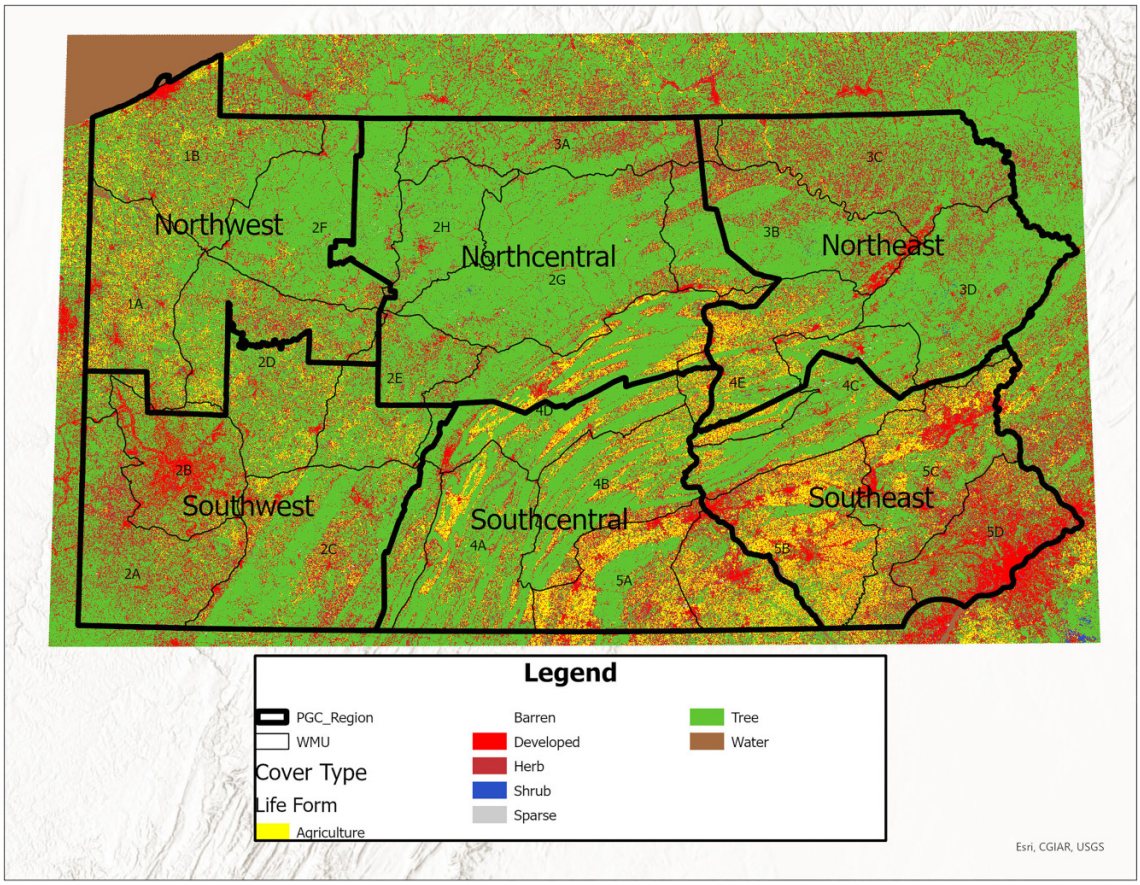
No live animals were handled by the researchers for this work.

Lab analysis

Liver samples were screened for first- and second-generation ARs: brodifacoum, bromadiolone, chlorophacinone, coumachlor, coumafuryl, dicoumarol, difenacoum, difethialone, diphacinone, pindone, and warfarin at the Pennsylvania Animal Diagnostic Laboratory System (PADLS) Toxicology Laboratory at New Bolton Center (Kennett Square, PA). A published QuEChERS extraction (short for quick, easy, cheap, effective, rugged, and safe) and high-performance liquid chromatography (HPLC) method (Vudathala et al. 2010) was used for the analysis of the livers. We established the method detection limit by using 1 g of liver which was spiked at 0, 0.01, 0.05, and 0.500 μg/g on a wet mass basis. One gram of liver was then homogenized with 4 mL of acetonitrile and centrifuged to form a soft pellet. The organic layer was transferred to a clean test tube containing 0.03 g PSA (primary–secondary amine), 0.10 g florisil adsorbent, 0.175 g $MgSO_4$, and 0.05 g basic alumina. An additional 0.050 g of C_{18} sorbent was used to obtain a clean sample suitable for analysis. The tubes were vortexed for 15 s, allowed to rest for 5 min, and then centrifuged at approximately 438 g for 5 min. The supernatant was then transferred to a clean test tube and reduced to dryness under a stream of nitrogen using a warm (60 ± 5 °C) water bath. The residue was dissolved in 0.50 mL of ion pair diluent and then vortexed. The solution was microfuged through a 0.22-micron SpinX filter and the clear filtrate was transferred into an autosampler vial fitted with a high recovery vial for analysis.

Anticoagulant rodenticide detection and quantification was performed using a Shimadzu (Kyoto, Japan) HPLC system, consisting of a CBM-20 A controller, SIL-20AC autosampler, AC-20AT pump, SPD-M20A diode array detector, RF-10AXL fluorescence detector, and a Betasil reverse phase C18 column 150 × 4.6 mm, 5 μm particle size (Thermo Electron Corporation). We conducted all HPLC analyses using 0.03 M tetrabutylammonium hydroxide (TBA) buffer (adjusted to pH 7 with o-phosphoric acid/methanol, 25:75, v/v) as solvent A and methanol as solvent B at a flow rate of 0.6 mL/min in a gradient run. We set the gradient at 30% B for 10 min followed by gradient to 80% B in 4.5 min, and increasing to 90% B in the next 0.5 min. We washed the column with 90% B for 5 min followed by equilibration to initial conditions for 4 min. The fluorescence detector was set at 280 nm excitation and 410 nm emission. We monitored the diode array detector from 240 to 340 nm, with quantification done at 325 nm. We confirmed our results in the abovementioned conditions and with solvents at a slightly different gradient. The gradient consisted of 30% B for 7 min, a gradient to 75% B in the next 3 min, increasing to 80% B in the next 11 min, then holding

Fig. 1. Pennsylvania Game Commission regions (thick black lines) and wildlife management units (thin black lines) with color background depicting eight broad land cover types across Pennsylvania. The spatial data used for the regional and unit lines were accessed through the Pennsylvania Spatial Data Access (PASDA 2022; https://www.pasda.psu.edu/) and cover types were downloaded through LandFire (2022) (https://landfire.gov/). All spatial layers were projected using Lambert Conformal Conic coordinate system and the North American Datum (NAD) 1983.



it for 5 min followed by equilibration to starting conditions for the next 4 min (Vudathala et al. 2010).

We used fluorescence detection to monitor for brodifacoum, bromadiolone, coumafuryl, difenacoum, and warfarin; UV spectra monitored for the presence of chlorphacinone, difethialone, and diphacinone. Each compound had an established limit of quantification (LOQ), which varied by compound as follows: brodifacoum (0.010 μg/g), bromadiolone (0.025 μg/g), chlorophacinone (0.050 μg/g), coumachlor (0.100 μg/g), coumafuryl (0.100 μg/g), dicoumarol (0.100 μg/g), difenacoum (0.010 μg/g), difethialone (0.050 μg/g), diphacinone (0.050 μg/g), pindone (0.100 μg/g), and warfarin (0.100 μg/g). Positive results below the LOQ were recorded as trace amounts. At or above the LOQ, numerical concentrations were recorded in parts per million (ppm), which is equivalent to micrograms per gram (μg/g), on a wet weight basis. For our analyses, we report any amount that was detectable, even those below LOQ, as a positive detection.

Statistical analysis

For most analyses, we used individual exposure rather than population-level exposure to assess patterns of AR distribution and detection. We included in our analysis all detected ARs (trace or quantifiable amounts) to estimate exposure. Where appropriate, we report concentration amounts in μg/g on a wet weight basis for samples that were above trace level. Using Statistical Application Software (SAS; Cary NC), we fit generalized linear models with a binomial distribution to determine if there were differences in detection proportion among the three species. Further, we examined if there were differences in AR detection among PGC regions (Fig. 1) and then we examined both an additive and interactive model using both species and region. We obtained data on landcover for the year 2020 from the Landfire website (landfire.gov) and used the "ENV_LF" categorization of these data (Fig. 1). This classification system places all land within PA as being forest, herbaceous, water, barren, developed, shrub, agriculture, or sparse. For our analyses, we considered the proportion of land in each PGC region and wildlife management units that were classified as developed and agricultural. We also added the values of the developed and agricultural land together to provide a metric that included those two land classes that we hypothesized were associated with rodenticide availability and ultimately detection in wild carnivore populations. For both PGC region and wildlife management units, we obtained shapefiles delineating borders from Penn-

Table 1. Number of samples with detectable anticoagulant rodenticide concentrations by compound types and the percentage of samples detected above the minimum detectable amount and (% of quantifiable compounds >0.1 μg/g on a wet weight basis) for those with quantifiable levels, their mean concentration (Mean Conc), and the minimum and maximum quantifiable concentrations (range) observed for all detections for three species of carnivores (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) in Pennsylvania from 2019 to 2022.

Compound	Detections	Percent > trace (percent > 0.1 μg/g)	Mean conc (μg/g)	Range
Warfarin	2	1.6% (0.0%)	–	0–0
Coumafuryl	0	0.0% (0.0%)	–	0–0
Diphacinone	59	47.9% (52.0%)	0.2	0.05–0.78
Pindone	0	0.00% (0.0%)	–	0–0
Brodifacoum	27	21.9% (16%)	0.05	0.01–0.25
Difenacoum	2	1.6% (0.0%)	–	0–0
Bromadiolone	8	6.5% (15.3%)	0.07	0.03–0.28
Chlorophacinone	8	6.5% (0.0%)	–	0–0
Difethialone	7	5.7% (1.0%)	0.16	0.16
Dicoumarol	10	8.1% (75.0%)	0.54	0.1–1.78
Coumachlor	0	0.0% (0.0%)	–	0–0

Note: Mean concentrations and standard deviations are not reported for compounds only detected at trace levels, below the method's limits of quantification.

sylvania Spatial Data Access (https://www.pasda.psu.edu/). For both wildlife management units and PGC region, we used the "Zonal Histogram" tool in ArcGIS Pro version 9.1 (ESRI Co, Redlands CA, USA) to quantify the total proportion of landcover types within each respective geographic unit. Finally, for each region, we calculated the proportion of AR detections across all three carnivore species and then for each species individually. For wildlife management units, we calculated the proportion of detections for all three species combined but could not do so for each species individually because we had insufficient data across all units.

Using generalized linear models, we tested for a positive relationship between the proportion of a land unit that was developed or agricultural, and finally a combination of developed and agricultural lands per region or wildlife management units and the proportion of samples where we detected ARs in those respective spatial units. At the regional scale, we evaluated AR detection across all three species in addition to each species individually. We recognized that the combined metric would be correlated to either, or both, developed and agricultural lands, but our intention was to evaluate whether the proportion of either developed or agricultural lands better explained patterns of AR detections for any species more robustly than a single cumulative metric. For these analyses, we used a gamma distribution and a log-link transformation. Finally, we evaluated whether the age of the animals was related to AR detections by using generalized linear models to test for a relationship between AR detection and age of the animals.

Results

We collected and generated AR results from 265 livers from three carnivore species in Pennsylvania. Most (65%) were collected from carcasses legally harvested by trappers. Mistakenly killed or illegally killed animals accounted for 16.6% of all carcasses, followed by road-killed animals (12.8%) and the remainder (5.6%) were killed or collected without documentation. We collected 105 (39.6%) river otters, 97 (36.6%) fishers, and 63 (23.7%) bobcats. We detected ARs at both trace and quantifiable levels in all three species examined. Across species, the total trace detection rate was 44.2%, whereas 17.4% had quantifiable levels of ARs. Of the 11 compounds for which we tested, only three were not detected in any liver sample (coumafuryl, pindone, and coumachlor) (Table 1). Diphacinone and brodifacoum were the most detected AR compounds across all species at both trace and quantifiable levels (Table 1). Bromadiolone and dicoumarol were detected at modest levels compared to other compounds. Thirteen individuals were found to have >2 compounds at quantifiable levels and 33 had a single quantifiable compound. One animal had trace levels of four different compounds, five had three, and 11 had two; all other trace detections were positive for one compound (Table 1).

Dicoumarol was found in the highest quantifiable concentrations (0.53 μg/g ± 0.82 SD) (Table 2). Generally, there was high variation among the concentrations found across individuals and species for both the numbers and types of compounds found. Species differences existed within the data at regional and wildlife management units levels. Statewide, species differences were detected ($\chi^2_{[2]} = 61.86$, $P < 0.0001$) and fishers had the highest exposure across the state with 70% of all fishers testing positive for at least one compound (Table 2). Bobcats had the second highest AR detection, followed by river otters (Table 2). Nearly half of all bobcats tested showed at least trace AR levels, whereas 17% of otters were exposed to ARs (Table 2). Evaluating regional differences in exposure to ARs across all species indicated high variation across regions (Table 3). We also had high variation in the numbers of samples we received from each region, with the Southeast providing 8 and the Northeast providing 79 samples, respectively. These differences may be attributed to dif-

Table 2. Total number of samples (Total), samples with any positive anticoagulant rodenticide detections (Detections), the percentage detection within species (Percent detected), and % of all detections (Percent species) pooled across species ($n = 117$) by species (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) for samples collected in Pennsylvania from 2019 to 2022.

Species	Total	Detections	Percent detected	Percent species
Bobcat	63	31	49.21%	26.50%
Fisher	97	68	70.10%	58.12%
River otter	105	18	17.14%	15.38%

Table 3. Total number of samples (Total) and positive anticoagulant rodenticide detections (Detections), and the percentage of samples with detections for each species (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) and region sampled in Pennsylvania from 2019 to 2022.

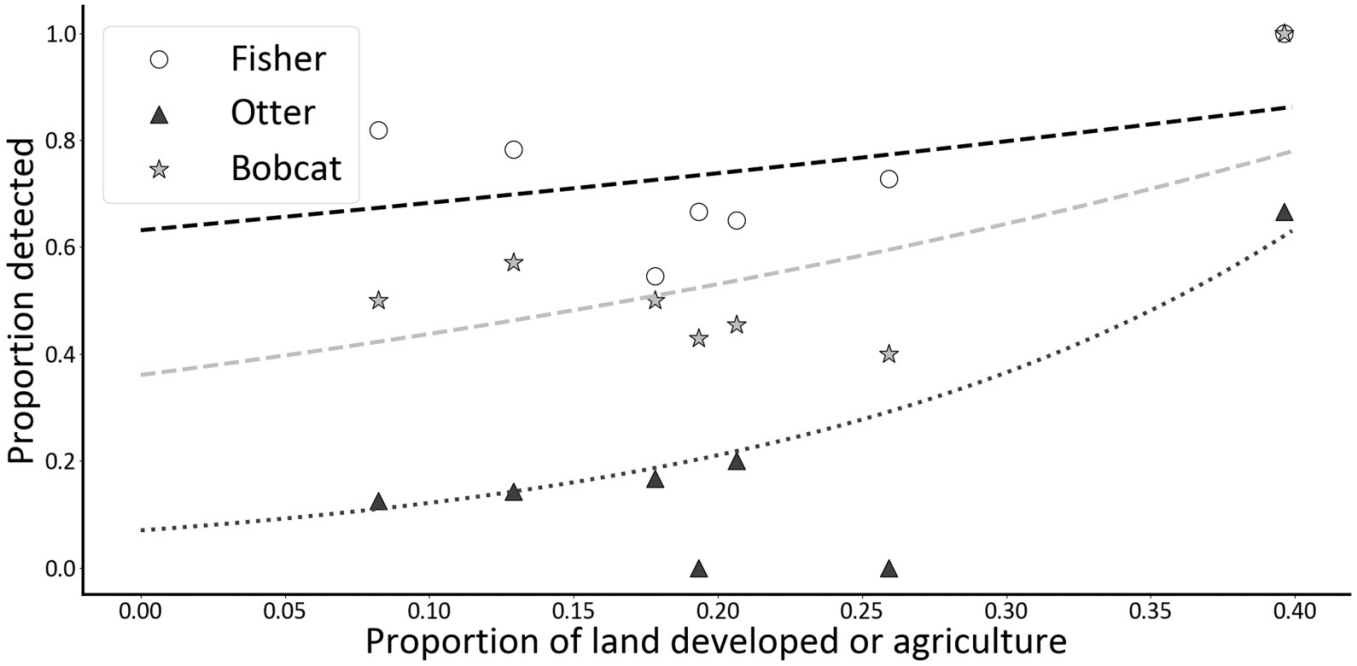
Region	Species	Total	Detections	Percent detected
North Central	Bobcat	16	8	50.0
	Fisher	11	9	81.8
	River otter	8	1	12.5
		35	18	51.4
North East	Bobcat	14	8	57.1
	Fisher	23	18	78.3
	River otter	42	6	14.3
		79	32	40.5
North West	Bobcat	4	2	50.0
	Fisher	22	12	54.5
	River otter	18	3	16.7
		44	17	38.6
South Central	Bobcat	10	4	40.0
	Fisher	11	8	72.7
	River otter	0	0	•
		21	12	57.1
South East	Bobcat	1	1	100.0
	Fisher	4	4	100.0
	River otter	3	2	66.7
		8	7	87.5
South West	Bobcat	7	3	42.9
	Fisher	6	4	66.7
	River otter	4	0	0.0
		17	7	41.2
Unknown	Bobcat	11	5	45.5
	Fisher	20	13	65.0
	River otter	30	6	20.0
		61	24	39.3
Total		265	117	44.2

ferences in species representations or simply due to random chance or collection effort. Nevertheless, regional differences did not provide a significant descriptor of detection ($\chi^2_{[6]} = 10.36$, $P < 0.11$). When we included both species and region in an additive model, we found a similar pattern where region had little explanatory power. Additionally, an interactive model did not converge because the data were too scant. On average, the SE region had the highest exposure of AR detection (mean = 87.5%) across all samples and species.

Regional landcover analyses

The regional analyses revealed that all regions are dominated by forested land cover (62% ± 12%). The SE region has the highest proportion of developed and agricultural land

Fig. 2. Regionally explicit relationships between anticoagulant rodenticide detections for bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*) in Pennsylvania from 2019 to 2022.



(21% and 17%, respectively). The SW region has the second highest proportion of developed lands (12%), whereas the Southcentral region has the second highest proportion of agricultural lands (16.9%) (Fig. 2).

There was a positive relationship between AR detections, using all species, and the proportion of land that was developed or agricultural ($\beta = 2.15 \pm 0.53$, $\chi^2_{[1]} = 6.41$, $P = 0.011$). Additionally, a similar pattern emerged when we considered individual species and used either proportion of land that was developed or agricultural as individual explanatory variables (Table S1). The lone exception was that fishers did not exhibit a positive relationship between AR detection and any land-cover metric we tested (Fig. 3; Supplemental Table S1). For each species, the region with the highest proportion of developed or agricultural land had the highest AR detection rates (Supplemental Fig. S2). The SE region, which has the greatest development and human population densities, is associated with higher proportions of AR exposure regardless of which dependent metrics we examined.

Wildlife managment unit analyses

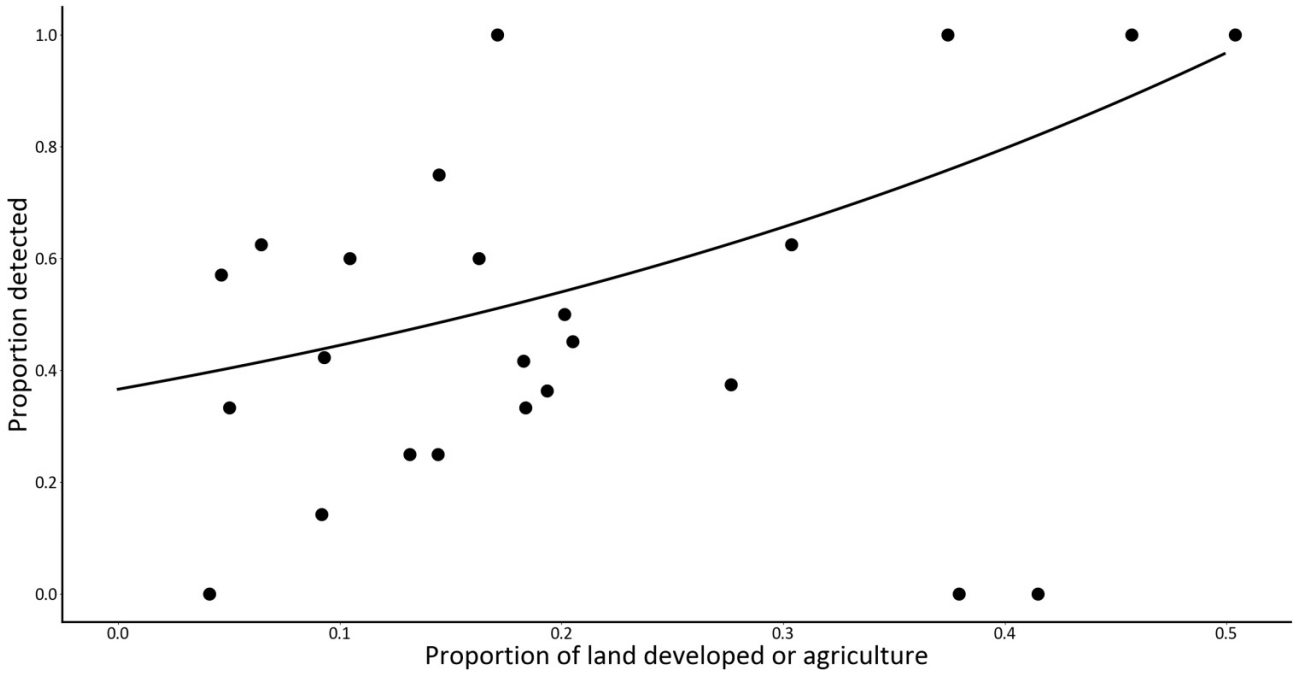
For the wildllife management unit analyses, we confined the analyses to AR detection across all species because we had insufficient data to perform these analyses for each species individually. Using the wildlife management unit-based values, we found that there was a positive relationship between the total detections per wildlife management units and the average concentration of ARs found ($\beta = 7.58 \pm 0.13$, $\chi^2_{[1]} = 6.8$, $P = 0.0089$). Generally, the distribution of land cover we found in the regional analyses was recapitulated in the wildlife management units analyses, but with greater replication at a finer scale. The wildlife management units in the Southeast (around Philadelphia) and Southwest (around Pittsburgh) have the highest proportion of developed and agricultural lands. Northern Wildlife Management Units have relatively undeveloped land and more tree cover. We found strong associations with developed or agricultural lands ($\beta = -2.394 \pm 0.1$, $\chi^2_{[1]} = 8.4$, $P = 0.0038$). The highest rates of detection were found in the Southeast and Southwest Wildlife Management Units (Fig. 3) In contrast, several wildlife management units with relatively low amounts of developed or agricultural lands had relatively high proportions of AR exposure across species, especially fishers.

Age analysis

We were able to estimate ages for 113 (42%) carcasses. Ages for the others could not be estimated either because the head was not present or because we could not extract a tooth. Across all three species, ages ranged from young of the year to 8 years. Of the individuals that had age data, 79% were 2 years or younger (Supplementary Table S2). With respect to species, 17 (73%) of bobcats, 32 (91%) of fishers, and 41 (74%) of otters were 2 years or younger. The oldest bobcats ($n = 2$) were 6 years old; a single fisher was 5 years old, and three otters were estimated to be 8 years old (Supplementary Table S2).

Age, across species, was not an important determinant of the probability that an animal would have any type of AR detection ($\beta = 0.127 \pm 0.009$, $\chi^2_{[1]} = 1.98$, $P = 0.15$). This result appears to be explained by species effects because only otters and bobcats were older than 5 years, and none of those animals tested positive for quantifiable levels of ARs. Only one

Fig. 3. Relationships between anticoagulant rodenticide detections for all detections for all species in Pennsylvania from 2019 to 2022.



fisher and one otter older than 4 years tested positive for ARs. When we tested models with both species and age effects, age was found to be unimportant ($\chi^2_{[1]} = 0.17$, $P = 0.67$). We tested additional models for age considering the individual species, but all failed to show significant results relative to patterns of age and AR exposure.

Discussion

Our study indicates that AR exposure among mesocarnivores is relatively common in Pennsylvania and that they are most detected in areas with high human development. Nevertheless, ARs appear to be present, and to some degree common, in even less human-dominated landscapes. ARs appeared in every species we examined. Our study appears to be the first to take multi-species mesocarnivore approach across a large geographic region. Pennsylvania is among the largest states in the NE United States, which provided us a unique ability to assess ARs within and across regions of different human population densities and land uses. Though our study supports other studies of exposure to ARs within carnivores, it is distinctive in comparing relative rates of exposure among similar sized carnivores with different life histories. Consequently, our study is the first to explicitly test hypotheses about mechanisms of exposure at broad scales. Additionally, our study provides a valuable baseline for AR exposure for three species of mesocarnivores in Pennsylvania. This baseline may be used to assess population trends in mesocarnivores and to build upon our understanding of the health and influence of habitats in which these and other species occur.

We found ARs across all six PGC regions of Pennsylvania and in all three species of carnivores we examined. Fishers were significantly more likely to be exposed to ARs regardless of region compared to bobcat and river otter. Bobcats had relatively higher rates of exposure compared to otters but lower than fishers (Table 2). Detection rates of ARs for fishers in Pennsylvania (70%) were similar, though modestly lower, to those in California (79%) (Gabriel et al. 2012). Yet, detection rates for fishers in Pennsylvania were nearly 30% lower than those detected in Vermont and New Hampshire (97%, $n = 45$) (Buckley et al. 2023). Based on liver samples, Pennsylvania bobcats also had lower rates of AR exposure (49%) compared to bobcats in Southern California (89%) (Serieys et al. 2015). Bobcats in southern California may live in proximity to higher human densities than across Pennsylvania. For example, AR exposure rates were also very high for a study of San Joaquin kit foxes (86%, $n = 30$) near Bakersfield, CA (McMillin et al. 2008). Had we acquired more samples from animals near urban areas, our results may have been similar. Critically, the studies in California, were based on long-term intensive studies of individual populations where animals were tracked and individual mortalities were investigated. Our study focused on animals we could sample coincidentally due to death from other means across the state. Such differences in study approach and intensity may contribute to differences in detection rates that we cannot account for nor quantify. The differences among studies, therefore, should not be taken as a conclusion about relative levels of ARs within those systems.

Importantly, we demonstrate that river otters, along with terrestrial mesocarnivores, were exposed to ARs in the eastern US, although the pathways that lead to exposure may be different or less prevalent than in fishers or bobcats. We have shown a single otter exposure above the method detection

limit for the compound brodifacoum (at 0.01 μg/g), which has not been previously documented in the primary literature. Other otter species and semi-aquatic mammal and bird species are known to be exposed in other systems and our study appears to confirm the presence of ARs in aquatic systems in Pennsylvania and likely throughout the Northeastern United States (Ruiz-Suárez et al. 2016; Serieys et al. 2019; Niedringhaus et al. 2021). In keeping with our general understanding of how carnivores are exposed to ARs, the highest rates of exposure in PA occurred in areas with relatively high rates of human development and agriculture, areas where rodenticides should have the highest use and highest likelihood for contact with wildlife species. We found that at the broad regional scales there were not differences in exposure rates for any species or for all species combined. We did see such differences at the level of the WMU and it indicates that even though exposure rates are high near cities and agriculture, there were still high rates of exposure in some portion of virtually every region.

We hypothesized fishers would have the highest rates of exposure compared to the other carnivores we tested because they are the species most likely to scavenge and prey upon AR-exposed rodents (Gabriel et al. 2012; Gabriel et al. 2015). Bobcats are less likely to scavenge in terrestrial systems but could conceivably capture sick or AR-weakened rodents. Nevertheless, because fishers would scavenge and eat these affected rodents, we expect them to have higher rates of exposure compared to bobcats. Otters forage and live in aquatic systems where less is understood about AR detection and exposure in mammals. Even so, having relatively low human development does not indicate rodenticides are absent from aquatic or terrestrial ecosystems. In our study, regions or wildlife management Units with high forest or non-developed areas still showed carnivore exposure to ARs, and this is likely due to the relative abundance of housing and agriculture throughout PA. More detailed information about carnivore home ranges, use of areas near housing, and sources of ARs is required to fully understand the mechanisms by which they are encountering, presumably, AR-exposed rodents or being exposed directly. Some ARs are flavored (e.g., bacon or cheese) such that they could be attractive to carnivores and consumed directly if they are not used appropriately. The relatively low rates, and concentrations, of exposure in otters could represent a modest AR signal in the aquatic systems (e.g., invertebrates and fish) or could represent exposure from terrestrial systems that ultimately find their way into the aquatic ecosystem. Like fishers, otters are mustelids that will scavenge carcasses, and AR-exposed rodents that wash downstream from terrestrial systems could be consumed. Large cities may also liberally use rodenticide within sewer systems, which could directly introduce ARs into the aquatic system (Regnery et al. 2020). All mechanisms are possible, or even likely, and the presence of ARs in otters cannot exclude any of these.

During our study, we collected more otter carcasses than we predicted and relatively fewer bobcats and fishers. We may have received relatively high numbers of otter samples because otters are inadvertently killed in beaver sets ($n = 29$ for this study) during the PA trapping season, and because all legal otter harvests ($n = 70$) must be reported to local game wardens to obtain a Convention on International Trade in Endangered Species (CITES) permit. Both circumstances provide additional contact with PGC wardens and increase the probability of obtaining samples. Though we did not expect that otters would have high rates of detection, the relatively large numbers of samples from otters do provide an important examination of their rate of exposure to ARs that has not previously been documented. The PGC also must issue a CITES tag for all legally harvested bobcats; however, the method for obtaining tags for bobcats is different from otters and does not place trappers in contact with game wardens under typical legal harvests where trappers simply self-report their harvest. Additionally, the species is less likely to be mistakenly killed in traps set for other species ($n = 2$ for this study). Permitted fisher and bobcat trappers are limited to one of each species per person per season and some trappers were reluctant to relinquish them for study (especially when we requested the head). For all three species, legal trapping occurs only in select portions of the state, and not the same portions for all species, because stable or increasing populations do not occur in all parts of the state. Consequently, there is some bias associated with where legally harvested samples come from with respect to regions and wildlife management units. Fewer animals live in regions without harvest, so there are also fewer opportunities to collect specimens from those regions even through other types of mortalities (e.g., vehicular collisions). At finer spatial scales (wildlife management units or county), more samples are needed to fully address the prevalence of ARs within the carnivore community and within individual species (see Table 3). Undoubtedly, spatial results based on only a few specimens may paint an incomplete picture of actual exposure rates. Future studies should attempt to address issues relative to sample sizes.

We found no strong association between exposure rates and age in the animals we tested. These results are also somewhat contingent on sample size, as we had relatively few animals that were over 2 years old (Supplementary Table S2). The lack of older animals in our sample may indicate that there are few animals in the older age cohorts or that older animals are inherently more wary and so less likely to be trapped. Alternatively, or in addition, the lack of old animals in our samples may be related to another issue. ARs at sublethal doses may negatively affect behavior, impair the decision-making of animals, or make them more risk-prone. Such behavioral effects from ARs could therefore make them more likely to be trapped, hit by cars, or to seek food closer to urban areas where they will be considered a nuisance (López-Perea et al. 2019). Young animals may also share many of these same characteristics, making them more prone to being trapped or killed, accidentally killed, or less effective predators causing them to rely on scavenging or killing less evasive prey (e.g., AR-exposed rodents). Thus, our estimates may be biased toward animals that have been exposed to ARs. Overcoming this limitation would require a non-biased sampling approach. This would involve some form of randomly killing mesocarnivores in the wild, which is not a method that is likely to be used for recovering these relatively elusive and highly managed carnivores

(Keller 2021). Smaller, though more intensive, studies on any of these species could provide more information on exposure and related risks to individuals based on AR exposure. In general, we think that to the degree our sample is biased toward animals that are exposed, the magnitude of the bias is similar across regions. Consequently, the patterns of exposure across PA, and other geographic regions, and the associations to development and agriculture (human population density) are likely robust. More generally, our results fit within our general understanding of where ARs should have the highest contact with wildlife and for which species we predicted a priori should be most exposed.

The exact routes of AR exposure, concentrations in the liver associated with fatalities, and sub-lethal effects on wildlife are still largely unknown. Some animals with high body burdens of ARs may appear relatively healthy, whereas others with relatively low concentrations suffered internal bleeding and ultimately death (Gabriel et al. 2015; Sainsbury et al. 2018). Trace levels of ARs could represent relatively long-ago exposures or relatively small recent ingestions. Additionally, presence of multiple ARs could be the result of one or multiple exposures. Clearly, minor differences exist among data types, but all show similar patterns of exposure across species, regions of PA, and underlying ecological drivers (e.g., association with human development).

Evidence of rodenticide exposure in mesocarnivores is an expected finding in areas of high development such as much of PA (Gabriel et al. 2015; Lohr 2018; López-Perea et al. 2019; Cooke et al. 2022). How sublethal exposures affect individuals and populations is still unknown and needs further study (Quinn 2019). Here we have provided a baseline estimate of exposure, which may be used as a comparison for future studies. In particular, if populations of these carnivores change, then examination of the rates and levels of exposure over time could indirectly implicate or exclude ARs as a mechanism leading to those changes. We assume that ARs have been prevalent in the northeastern United States for at least 50 years. During that time, fishers and otters have been reintroduced to PA and both have shown relatively rapid expansion and population increases (Keller 2021). In particular, fishers seem to continue to thrive and expand into more urban landscapes, which would not be expected if ARs were strongly limiting populations. We cannot estimate how rodenticides may have reduced or minimized this apparent rate of growth and expansion, but ARs appear not to have prevented growth of the populations for any of the species we studied. Coincidentally, regions within PA having relatively low populations of fishers, bobcats, and otters (Keller 2021) also have relatively high rates of AR exposure. Nevertheless, those regions also have less productive habitats for all three species and ARs are confounding the absence of habitat that also occurs within those areas. Cross-regional examination of both AR exposure rates and changes in populations may further elucidate the possibility that these compounds have affected carnivore populations in PA and the eastern US. Future research concerning all three species could focus on diversifying sampling efforts outside of only harvested individuals and ensuring samples are evenly distributed throughout all regions and wildlife management units of the state. Sampling size could be increased to eliminate potential bias due to age, spatial scale, and cause of mortality. These steps may result in a more accurate estimation of detection levels throughout the state, their sources relative to carnivores, and their ultimate effect on populations. Research identifying point source, although challenging, will be beneficial to the resource. Research specific to river otters could focus on environmental sampling, including water, soil, and prey items such as fish, invertebrates, reptiles, and amphibians. Future monitoring for exposure to ARs in all three species could suggest changes in the abundance of rodenticides in these three species but may also serve as sentinels for other species of carnivores, raptors, or ecosystems in general.

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Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Competing interests

We have no competing interests to declare.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2023-0131.

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From: Mourad Gabriel <mgabriel@iercecology.org>
Sent: Thursday, November 6, 2025 9:30 AM
To: lowensvi@icloud.com; Jonathan Evans <JEvans@biologicaldiversity.org>
Subject: Re: Letter to DPR

Hello Lisa and Jonathan, I needed to run this by the other PIs on this project and see if all of the ducks were in a row. What could be added is below.. Is this helpful?

"Current data from investigations into the exposure to ARs in barred owls in Northern California demonstrate continued exposure to anticoagulant rodenticides throughout that landscape. Specifically, Second Generation AR (SGAR) are still being detected in barred owls, varying in age (1-10+years) throughout the Northern California landscape. Specifically, SGAR makes up the majority of exposures, 36% of over 700 owls collected and tested from 2018-2024."

This was presented by IERC at the North Coast Wildlife Society Science Conference in 2024.

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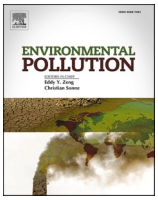

Collateral damage: Anticoagulant rodenticides pose threats to California condors☆

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ABSTRACT

Anticoagulant rodenticides (ARs) are widespread environmental contaminants that pose risks to scavenging birds because they routinely occur within their prey and can cause secondary poisoning. However, little is known about AR exposure in one of the rarest avian scavengers in the world, the California condor (*Gymnogyps californianus*). We assessed AR exposure in California condors and surrogate turkey vultures (*Cathartes aura*) to gauge potential hazard to a proposed future condor flock by determining how application rate and environmental factors influence exposure. Additionally, we examined whether ARs might be correlated with prolonged blood clotting time and potential mortality in condors. Only second-generation ARs (SGARs) were detected, and exposure was detected in all condor flocks. Liver AR residues were detected in 42% of the condors (27 of 65) and 93% of the turkey vultures (66 of 71). Although concentrations were generally low (<10 ng/g ww), 48% of the California condors and 64% of the turkey vultures exposed to ARs exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww), and 10% and 13% exceeded the 20% probability of exhibiting signs toxicosis (>80 ng/g ww). There was evidence of prolonged blood clotting time in 16% of the free-flying condors. For condors, there was a relationship between the interaction of AR exposure index (legal use across regions where condors existed) and precipitation, and the probability of detecting ARs in liver. Exposure to ARs may complicate recovery efforts of condor populations within their current range and in the soon to be established northern California experimental population. Continued monitoring of AR exposure using plasma blood clotting assays and residue analysis would allow for an improved understanding of their hazard to condors, particularly if paired with recent movement data that could elucidate exposure sources on the landscape occupied by this endangered species.

1. Introduction

Anticoagulant rodenticides (ARs; first- and second-generation rodenticides; hereafter FGAR and SGAR) have been used for decades to manage rodent pest populations, but can pose physiological risks to raptors and scavenging birds through secondary poisoning (Rattner et al., 2014a; Elliott et al., 2016; Hong et al., 2019) because they accumulate in their prey (e.g., *Rattus*; Elliott et al., 2014; Poessel et al., 2015; Geduhn et al., 2016). Secondary poisoning of birds can produce a range of deleterious physiological effects (e.g., anemia, hemorrhage, pallor of mucus membranes, depressed mentation and weakness), including direct mortality (Kelly et al., 2014a; Rattner et al., 2014a; Elliott et al., 2016; Hong et al., 2019; Rattner and Harvey, 2021). The widespread legal application of ARs, coupled with potential off-label illegal use, are the principal sources to wildlife (Gabriel et al., 2012; Elliott et al., 2014; Rattner et al., 2014b; Series et al., 2015).

Raptor exposure to ARs is globally widespread. A recent review (Elliott et al., 2016) found that average AR incidence (percentage of bird

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livers with at least one detectable SGAR) was 63% across 14 published studies (n = 2565 raptors) in nine countries. Within North America, SGARs are among the most frequent and abundantly used AR group (Murray, 2017; Quinn et al., 2019; Niedringhaus et al., 2020) and are routinely detected in wildlife because of their longer half-lives (Erickson and Urban, 2004; Herring et al., 2017). Birds that consume SGAR-poisoned animals have a higher likelihood of secondary poisoning and bioaccumulation compared to those exposed to first-generation ARs because of differences in AR toxicity and bioaccumulative potential (Murray, 2011; Thomas et al., 2011; Kelly et al., 2014b). Additional studies have shown that the degree of scavenging in raptors and other avian scavengers is positively correlated with AR exposure and mortality (Hughes et al., 2013; Hong et al., 2019).

The occurrence of ARs within raptor and avian scavenger food chains increases the likelihood of non-target AR exposure and potential adverse effects in those birds. The California condor (*Gymnogyps californianus*), a federally endangered scavenger, is one such species that is routinely exposed to contaminants through their food base (e.g., Pb; see Church et al., 2006; Finkelstein et al., 2012; Kelly et al., 2014a). California condors may consume ARs because they forage on agricultural pests and mammalian predators that have been exposed to ARs by primary or secondary routes, similar to other large facultative and obligate scavengers (e.g., golden eagle *Aquila chrysaetos*, turkey vulture *Cathartes aura*; Langford et al., 2013; Kelly et al., 2014b). With a global population of approximately 334 individuals, any additional physiological stressors could adversely affect their population. Therefore, identifying and understanding external factors that influence condor mortality or impairment are important for effective management of the current population, evaluating potential new release sites, and developing effective public outreach to protect the population.

We evaluated AR exposure in California condors across their current range in the central/southern California and Arizona flocks, as well as in a surrogate avian scavenger (turkey vultures *Carthartes aura*) in both existing condor range and at the future condor release site designated by the Northern California Condor Restoration Program. We determined AR exposure by sampling liver (long-term exposure: months) from deceased condors, blood (recent exposure: days – weeks) from free-ranging live condors, and liver and blood samples from surrogate turkey vultures. We then examined how legal AR application and environmental factors (e.g., precipitation) influenced variability in condor AR exposure and liver tissue concentrations, and whether ARs may be related to condor mortalities. We also assessed potential condor physiological responses to AR exposure, through common biomarker responses, prothrombin time (PT) and Russell's viper venom time (RVVT), to detect coagulopathy. These biomarkers can be used to demonstrate both the physiological response to ARs and as an indication of AR exposure (Rattner et al., 2014b; Rattner et al., 2015; Hindmarch et al., 2019).

2. Methods and study area

2.1. Quantify plasma and liver AR exposure in live and deceased California Condors throughout their current foraging range

California condors are captured on a semi-annual (typically 2–3 times) basis at Pinnacles National Park (PINN; Fig. S1) and undergo regular blood sampling to check health indices and measure exposure to environmental contaminants, particularly lead (Pb). During 2017–2018, a subset of blood samples was collected for AR analysis (n = 44) and blood clotting time biomarkers by venipuncture of the tarsal vein. Samples for AR analysis were stored in ethylenediaminetetraacetic acid (EDTA) blood tubes, and samples for clotting time biomarker testing were collected in citrated blood tubes; tubes were stored on ice and centrifuged at the end of the day (15 min at 2500 g) and plasma fractions were pipetted into individual cryovials (see Rattner et al., 2015) for details). The EDTA and the citrated plasma samples were frozen at −20 °C and shipped on dry ice within one day to the laboratory where they were stored at −80 °C. Condor liver samples (n = 65) from 2006 to 2018 that had been collected as part of the necropsy of deceased condors were requested from research laboratories that were holding samples. Deceased condors were from the PINN/Ventana Wilderness Society (n = 24), southern California (n = 33), and Arizona flocks (n = 8). All liver samples were kept at −80 °C until AR analysis. Additional citrated blood plasma samples (n = 10) were collected under the above protocols from captive condors at the Oregon Zoo to serve as reference coagulation samples by Oregon Zoo staff in 2018. All condor field protocols were covered by endangered species permit TE157291-1 and approved National Park Service Institutional Animal Care and Use Permit PWR-PINN-Condor-2016.A3, and turkey vultures sampling occurred under state (California: 010619, SC-4741; Oregon: 052-17, 094-19) and Federal permits (MB28361A-0, 09379, 21417) and an approved Institutional Animal Care and Use Permits (Protocol 08/09.W.89.A, 2017-001).

2.2. Quantify plasma and liver AR exposure in Turkey vultures within the potential foraging range of northern California Condors

To gain insight into potential condor AR exposure within current condor range as well as potential risk to the future northern California flock (Fig. S1), we collected turkey vultures (n = 71) as surrogates within the current California condor range near Pinnacles National Park (n = 16) and in northern California (n = 20) and southern Oregon (n = 35) using a 12-gauge shotgun and steel shot in 2018 and 2019. This approach allowed us to compare the concentrations and types of ARs to which condors and surrogate scavengers were exposed within the same spatial/temporal period. We collected whole blood (n = 48) from each sacrificed vulture using 20-25-gauge heparinized needles by cardiac puncture. Blood was centrifuged immediately after sampling (15 min at 2500 g), and plasma fractions were pipetted into individual cryovials. All vulture carcasses and plasma were frozen on dry ice in the field and held in a −20 °C freezer until being transferred to either a −20 °C or −80 °C laboratory freezer (carcasses and plasma respectively). We utilized additional live turkey vulture plasma samples (n = 23) collected between 2009 − 2013 (stored at −80 °C) by the Yurok Tribe Wildlife Department (see West et al., 2017 for collection details, Fig. S1).

2.3. Tissue sampling and rodenticide analysis

For both condors and turkey vultures, we examined the frequency of AR exposure based on detecting at least one AR in the liver and or blood plasma (Rattner et al., 2014a; Gabriel et al., 2018). Liver tissue from each condor or vulture was excised and homogenized in liquid nitrogen using a cryomill (SPEX SamplePrep, Metuchen, New Jersey), and an approximately 5 g aliquot was used for quantitative AR analysis. With long term frozen storage, moisture often sublimates from biological tissues, resulting in variable % moisture content. This can influence contaminant concentrations when analysis and reporting are done on a wet weight (ww) basis. We addressed this issue by freeze-drying a subsample of each liver sample to determine moisture content. Final liver AR concentrations were adjusted from ww to dry weight (dw) using the individual percent moisture content. To facilitate comparisons of liver AR concentrations data published in ww, we approximated liver fresh ww concentrations by back calculating ww concentrations using a fresh liver moisture content derived from turkey vultures (mean = 69.9 ± 0.5%, n = 71). Liver tissue aliquots and plasma of condors and vultures were analyzed by the Texas A&M Veterinary Medical Diagnostics Laboratory (College Station, Texas) for quantification of eight ARs, including 4 first-generation ARs (chlorophacinone, coumatetralyl, diphacinone, warfarin), and 4 second-generation ARs (brodifacoum, bromadiolone, difenacoum, difethialone). Rodenticides were quantified by high-performance liquid chromatography-tandem mass spectrometry using an Agilent 1200 series C and 6400 triple quad system (Series et al.,

2015). Plasma samples (~1 ml) were analyzed for ARs on a ww basis. Quality control blanks all reported zero ARs detected, analytical spike recovery averaged 107.7 ± 6.0% (n = 8), and the limit of quantification (LOQ) across all ARs averaged 5.1 ± 0.1 ng/g ww for liver and 2.1 ± 0.4 ng/g ww for plasma and limit of detection (LOD) was between 0.5 and 1.0 ng/g ww for liver and 0.2–0.4 ng/g ww in plasma. If an AR was detected in a sample, but below the LOQ and ≥ the limit of detection (LOD; the lowest concentration in a sample that could be detected but not necessarily quantified as an exact concentration), we used the reported concentration rather than a ½ LOD which is commonly used but has no statistical underpinning (Zoffoli et al., 2013). Across all birds, only SGARs were detected, and as such, the molecular weights were generally similar and summing ARs is less problematic than if we had detected both FGARs and SGARs, which have vastly different molecular weights, toxic potencies and tissue half-lives (Horak et al., 2018; Rattner and Harvey, 2021). In all subsequent analyses, we summed SGARs (ΣSGAR) to simplify interpretation.

Physiological Biomarkers in California Condors and Surrogate Obligate Avian Scavengers.

Prothrombin time and RVVT of citrated California condor plasma samples (n = 47) were used to evaluate evidence of physiological response to potential AR exposure. Thrombin clotting time (TCT) was used as an indicator of fibrinogen concentration in plasma samples. Fibrinogen formation is insensitive to deficiency of vitamin K-dependent clotting factors caused by ARs, but its deficiency resulting from improper blood sample collection can prolong clotting time and confound AR toxicity studies (Rattner et al., 2010). Thus, it is important to verify that fibrinogen concentration is adequate to promote clot formation (~75 mg/dL). Reagents, conduct, and performance of these assays in various species of raptors has been previously described (e.g., (Rattner et al., 2011; Rattner et al., 2015; Hindmarch et al., 2019). The mechanical clot endpoint in these assays was determined using a Start4 fibrometer (Diagnostica Stago Inc., Parsippany, NJ). Nearly all samples were assayed in duplicate (a few [see summaries below] had inadequate volume and were assayed as a single determination), and values were averaged.

For the TCT assay, condor samples were run over a two-day period. Of the 47 samples assayed, 46 produced clots, and of those analyzed in duplicate (n = 44), the average coefficient of variation (CV) ± standard deviation (SD) was 4.1 ± 6.0%. Nine aliquots derived from a pool of citrated chicken plasma were assayed at various intervals during the two-day period to verify assay performance and yielded an average CV ± SD of 2.0 ± 1.8%. For the RVVT assay, there was adequate sample volume for analysis of 46 of the 47 samples and they were analyzed in a single day. Of the 42 condor samples assayed in duplicate, the average CV ± SD was 0.7 ± 0.6%. Seven aliquots derived from a pool of citrated chicken plasma were assayed for RVVT at various intervals and yielded a CV ± SD of 0.7 ± 0.9%. For the PT assay, there was adequate sample volume for analysis of 46 of the 47 samples and they were analyzed in single day. Of the 42 condor samples assayed in duplicate, the CV ± SD was 2.4 ± 3.0%. Seven aliquots derived from a pool of citrated chicken plasma were assayed for PT at various intervals and yielded a CV ± SD of 2.6 ± 1.4%.

2.4. Landscape analysis

To understand the potential influence of landscape variables on condor AR exposure and concentrations, we used ArcMap 10.4.1 (ESRI, Redlands, California, USA) to quantify precipitation and SGAR use (application rates) associated with the region in which a condor either was found deceased or was using prior to mortality. We restricted the time frame for the spatial analysis of each condor to the one-year period immediately prior to death. We selected this time frame because it represents a plausible approximation of when AR exposure would have occurred based on their half-lives in liver (Erickson and Urban, 2004; Herring et al., 2017). We did not assess the influence of landscape factors on AR exposure and concentrations for condors in Arizona because there are no available AR application data of adequate spatial resolution. Some deceased condors (70%) were not equipped with Global System for Mobile Communications (GSM)/Global Positioning System (GPS) transmitters, precluding us from utilizing individual level movement data for each bird. Rather, we used all available location data from other condors within the flock from which the deceased condor was associated during the one-year period prior to its death. We utilized data from Movebank (www.movebank.com) to understand spatial movement patterns for each condor in the central and southern California flocks. We determined the proportion of location detections per county relative to the total number of location detections for all GSM/GPS transmitted condors across the one-year period. We verified the viability of estimates of the percent of time spent in each county through correlation between estimates from all condor movement data and instances where we had actual data from individual condors ($F_{1,6.80} = 104.38$, $P < 0.0001$, $R^2 = 0.37$).

We developed an index of potential condor AR exposure based on both county level AR application rates and the proportion of time condor flocks spent in each county (hereafter AR exposure index). This was accomplished employing legal county-specific data on pesticide use (California Department of Pesticide Regulation, 2020) to estimate total AR application rates within each county for the year prior to each condors' death. We selected only those ARs that condors were exposed to as determined by our liver exposure data (i.e., brodifacoum, bromadiolone, and difethialone; see Results). For each county, total active ingredient (kg) of all ARs applied were summed across the year prior to mortality and adjusted for the area of the county (i.e., weighted average).

Rodent control is one of the primary drivers of AR use (Rattner et al., 2014a; Elliott et al., 2016). As a result, AR use fluctuates with rodent population cycles (Luque-Larena et al., 2013; Lopez-Perea and Mateo, 2018). Additionally, precipitation is a major factor influencing rodent populations (Brown and Heske, 1990; Meserve et al., 2003; Gillespie et al., 2008), so we used county-specific precipitation measurements for the rainy season (Oct–April) prior to each condors' death as a proxy for potential changes in county level rodent populations, which may have influenced AR application (both legal and illegal). Total county level precipitation was calculated from the PRISM Climate Group (2020). Total precipitation (mm) for each county were summed across each rainy season and adjusted to account for the proportion of time each condor flock spent in that county.

3. Statistical analysis

3.1. AR exposure

We evaluated factors influencing condor and vulture AR exposure using species-specific logistic regression models. Each bird's classification as "exposed" (at least one quantifiable AR detected) or "unexposed" (failure to detect ARs in liver) was used as the response variable. We then tested the probability of various factors influencing AR exposure using a logistic regression model with sex, region, and year of death as independent variables. For condors, we also included the AR exposure index and rainy season precipitation prior to the condor's death, along with the interaction between AR exposure index and precipitation to determine if precipitation influenced the relationship between AR exposure in condors and AR exposure index. We did not include an AR exposure index or precipitation in vulture models because no location data existed to define the spatial area covered by vultures. Regions for condor analysis were aligned with condor flocks and are based on GSM/GPS telemetry movement data (Southern California flock: Kern, Los Angeles, Santa Barbara, Tulare, and Ventura counties; Central California flock: Monterey, San Benito, and San Luis Obispo counties). Turkey vulture regions were associated with collection sites (Pinnacles National Park, northern California, and southern Oregon). We combined the

Pinnacles and Ventana flocks for analysis because of their propensity to move and feed together on the landscape (Bakker et al., 2017). Year was not included in vulture model because we did not sample vultures in each region every year.

To evaluate the factors influencing condor and vulture AR concentrations in birds with quantifiable AR concentrations, we used species-specific linear mixed-effects models. Liver AR concentration (ΣSGAR ng/g dw) was the response variable, with AR exposure index, precipitation, sex, region, and year of death as independent variables, and an AR exposure index × precipitation interaction. We included sampling site as a random effect in the turkey vulture models to avoid confounding effects associated with sampling multiple birds from the same location, whereas condor samples were considered independent because of the spatial and temporal differences in deaths.

3.2. Influence of AR exposure on condor cause of death

To assess if ARs influenced the probability of condors dying from a specific cause, we used cause-of-death determinations determined by necropsy by the California Condor Recovery Program and the U.S. Fish and Wildlife Service National Forensics Laboratory (see Viner et al., 2020 for details). Most condor mortalities were the result of either Pb poisoning (67%) or a range of other causes that were not numerous enough to be analyzed individually (4%–7% per cause of death). Therefore, we combined data for other causes of death (e.g., drowning, electrocution, entanglement, trauma) in a single category, thereby categorizing cause-of-death as either (1) "Pb toxicosis", or (2) "other causes". We then tested whether ARs influenced the probably of death via Pb toxicosis or other causes using a logistic regression model with liver ΣSGAR concentration (ng/g dw) and sex as independent variables. We included a liver ΣSGAR × sex interaction to determine if sex influenced the relationship between ΣSGAR concentrations and cause-of-death. Across all models we natural log-transformed all ΣSGAR, AR exposure index, and precipitation data to improve normality of the residuals and homogenize the variance structure.

3.3. AR biomarker response

To evaluate physiological responses to AR concentrations, we used general linear-mixed effects models to contrast fibrinogen concentrations, PT and RVVT in free-flying condors versus captive condors. We included condor ID as a random effect to avoid confounding effects associated with sampling the same condor on multiple occasions. For free-flying condors, we considered AR exposure to be indicated by physiological response values outside the upper extremes for control condors, following the general guidelines that prolongation of prothrombin times by two standard deviations above the arithmetic mean is suggestive of AR exposure (Shlosberg and Booth, 2006; Hindmarch et al., 2019).

4. Results

In this study, only second-generation AR compounds were detected. There were no AR detections in the 44 California condor plasma samples, but SGARs were present in 10% (5/48) of turkey vulture plasma samples (Table 1). Of the five turkey vultures with detectable SGARs in plasma, only one had a concentration above the limit of quantification (3.40 ng/g ww), whereas the other four had trace levels (<1.40 ng/g ww; Table 1). All five of these vulture plasma samples contained brodifacoum, and one also contained bromadiolone, and another contained difethialone. In contrast to blood plasma, SGARs were detected in liver of 42% (27/65) of the condors and 93% (66/71) of the turkey vultures (Table 1). Liver ΣSGAR concentrations ranged from 4.0 to 466.7 ng/g dw (estimated ww range 1.2–135.5 ng/g) in condors and 3.4–932.8 ng/g dw (estimated ww range 0.9–287.8 ng/g) in vultures (Table 1). The geometric mean (± standard error) liver ΣSGAR concentrations were 57.4 ± 16.1 ng/g dw (estimated ww 17.3 ± 4.8 ng/g) in condors and 81.5 ± 13.1 ng/g dw (estimated ww 24.5 ± 3.9 ng/g) in vultures (Table 1). Of the condor livers with detectable concentrations of SGARS, 56% had brodifacoum, 30% had bromadiolone, and 4% had difethialone. Four detectable SGARs were measured in turkey vulture livers – 97% had brodifacoum, 45% had bromadiolone, 17% had difethialone, and 3% had difenacoum. Of the California condors and turkey vultures that had detectable concentrations of ARs, 48% and 64%, respectively, exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww (Thomas et al., 2011);), and 10% and 13%, respectfully, exceeded the 20% probability of exhibiting signs of toxicosis (>80 ng/g ww; Thomas et al., 2011).

4.1. Variables influencing AR exposure

There was a significant interaction between the AR exposure index and precipitation ($\chi^2_1 = 4.91$, P = 0.03; Fig. 1), indicating that the relationship between the likelihood of condor exposure to SGARS and AR exposure index was influenced by precipitation. However, the probability of detecting SGARs in condor livers was neither related to sex ($\chi^2_1 = 0.18$, P = 0.67), flock ($\chi^2_2 = 3.21$, P = 0.07), nor death year ($\chi^2_1 = 1.53$, P = 0.22). To facilitate interpretation of the interaction on the probability of condors having detectable concentrations of SGARs in their livers, we plotted the conditional slope coefficients for the effect of AR exposure index on the probability of condors having detectable concentrations of ARs across the range of the precipitation. This illustrates that the magnitude and direction of the relationship between AR exposure index and the probability of condors having detectable concentrations of SGARs changes depending upon the amount of precipitation. With very low precipitation (<150 mm) the effect of AR exposure index on the probability of detecting SGARs in condor livers is marginally negative, and as rainfall increase that relationship is neutral, but with elevated rainfall (>460 mm), that relationship becomes positive (Fig. 1). Turkey vulture AR concentrations did not differ among regions ($\chi^2_2 = 0.35$, P = 0.84) nor by sex ($\chi^2_1 = 0.56$, P = 0.45).

4.2. Variables influencing AR concentrations

California condor liver ΣSGAR concentrations were positively correlated with year of death ($F_{1,12} = 4.77$, P = 0.05 (Fig. 2), but were not correlated with the AR exposure index ($F_{1,12} = 0.85$, P = 0.38), precipitation ($F_{1,12} = 1.04$, P = 0.33), region ($F_{1,12} = 0.04$, P = 0.85), sex ($F_{1,12} = 3.83$, P = 0.07), nor the AR exposure index × precipitation interaction ($F_{1,11} = 0.00$, P = 0.97). Turkey vulture liver ΣSGAR concentrations were not influenced by region ($F_{2,1.79} = 6.46$, P = 0.15) or sex ($F_{1,59.06} = 0.52$, P = 0.47).

4.3. Influence of AR exposure on condor cause of death

Liver ΣSGAR concentrations were positively associated with the likelihood of Pb toxicosis being the proximate cause of condor death ($\chi^2_1 = 3.92$, P = 0.05) and probability of condors succumbing to Pb toxicosis with increasing ΣSGAR concentrations was higher in female than male condors ($\chi^2_1 = 4.58$, P = 0.03). However, the 95% confidence intervals for both the ΣSGAR and sex odds ratios overlapped 1 slightly (odds = 2.49 [0.88–7.02] and 11.96 [0.94–151.77], respectively) indicating uncertainty around the effects likely due our limited sample size. Geometric mean ΣSGARs were on average 2.3-fold higher in condors that died of lead poisoning than condors that died of other causes (78.3 versus 34.5 ng/g dw, respectively).

4.4. AR biomarker response

Fibrinogen concentrations were 29% higher ($F_{1,35.77} = 5.21$, P = 0.03) in captive condors than in free-flying condors ($F_{1,35.19} = 4.82$, P = 0.03; Fig. 3A), but there were no differences in prothrombin time or

Table 1
Second-generation anticoagulant rodenticide (SGAR) residues in plasma (ng/g ww) and liver (ng/g ww unless specified) of California condors and turkey vultures. Condor samples were collected as part of ongoing health monitoring or from deceased condors (liver) throughout the current free-flying condor range with the United States (Arizona = AZ, Pinnacles NP/Ventana Wildlife Society = PINN/VWS, southern California = SCAL). Turkey vultures were sampled as a surrogate for condors within the current range of condors near Pinnacles National Park (PINN) or in northern California (NCA) and southern Oregon (SOR). Values below the limit of quantification are reported as "trace" and ND refers to not detected.

Species	Region	Tissue	*n*	Overall SGAR detection frequency %	Brodifacoum		Bromadiolone		Difethialone		Difenacoum		Geomean ΣSGARs (ng/g dw)	Geomean ΣSGARs (ng/g ww)
					% Positive[a]	Geomean (range)	% Positive	Geomean (range)	% Positive	Geomean (range)	% Positive	Geomean (range)		
California condor	All	Plasma	44	0	ND	–	ND	–	ND	–	ND	–	–	–
		Liver	65	42	56	16.3 (4.2–117.8)	30	11.2 (1.2–79.0)	4	89.1	ND	–	57.4	17.3
	AZ	Liver	8	25	100	7.7 (4.2–14.0)	ND	–	ND	–	ND	–	25.5	7.7
	PINN/ VWS	Liver	24	50	33	12.1 (8.0–27.0)	50	12.8 (3.3–79.0)	ND	–	ND	–	53.0	16.0
	SCAL	Liver	33	39	69	22.1 (5.6–117.8)	15	7.5 (1.2–47.4)	8	89.1	ND	–	70.8	21.3
Turkey vulture	All	Plasma	48	10	80	trace	20	1.9 (trace-1.9	20	1.4 (trace-1.4)	ND	–		3.4
	NCA	Plasma	23	0	0	–	ND	–	ND	–	ND	–	–	–
	PINN	Plasma	14	56	44	trace	11	trace	11	trace	ND	–	–	1.7
	SOR	Plasma	11	0	0	–	ND	–	ND	–	ND	–	–	–
	All	Liver	71	93	97	19.2 (trace-252.6)	45	9.1 (1.3–119.9)	17	6.3 (1.5–60.1)	3	3.0 (trace-12.7)	81.5	24.5
	NCA	Liver	20	95	95	6.6 (trace-43.7)	50	8.2 (2.3–54.5)	ND	–	ND	–	37.7	11.4
	PINN	Liver	16	94	93	42.1 (4.4–252.6)	53	63.4 (4.7–119.9)	20	10.3 (2.0–60.1)	7	12.7	152.9	46.1
	SOR	Liver	35	91	100	24.8 (1.8–167.4)	38	6.1 (1.3–33.9)	25	5.3 (1.5–12.1)	3	trace	95.4	28.8

[a] % Positive is the proportion of samples that tested positive for a specific AR divided by the total number of samples positive for ARs.

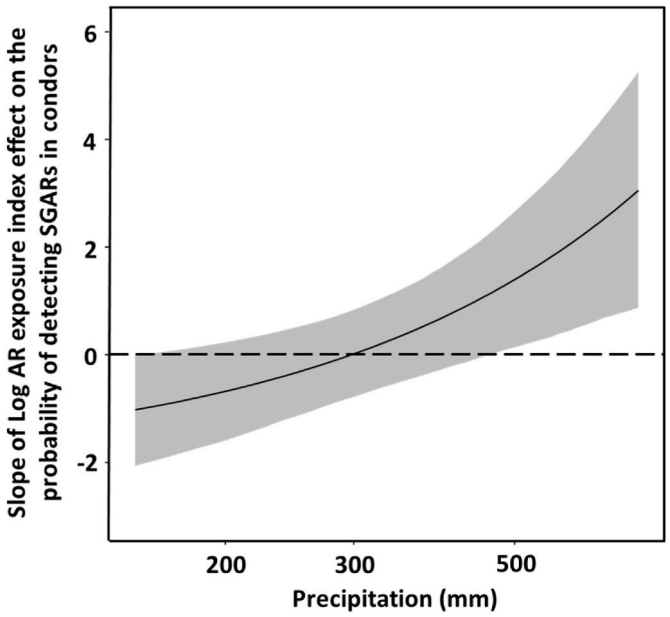

Fig. 1. Conditional effects of precipitation (mm) on the estimated coefficient of AR exposure index on the probability of California condors (*Gymnogyps californianus*) having detectable concentrations of SGARs in their livers after accounting for year of death, location, and sex. Shaded areas indicate the 95% confidence intervals around the estimated coefficient. The horizontal dashed line indicates a zero-coefficient value.

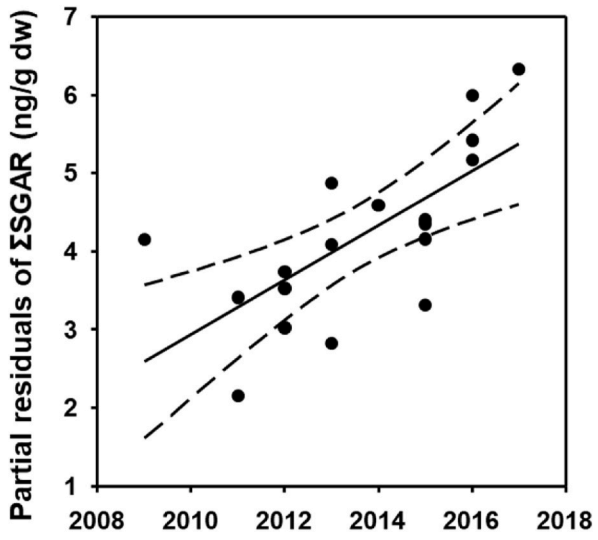

Fig. 2. Partial residual plot of California condor (*Gymnogyps californianus*) ΣSGARs (ng/g dw) and year of California condor death, accounting for the AR exposure index, precipitation, region, and sex. Dashed lines indicate the 95% confidence interval.

Russell's viper venom time between captive and free-flying condors ($F_{1,40.67} = 1.88$, $P = 0.17$) and ($F_{1,40} = 0.09$, $P = 0.76$), respectively (Fig. 3B and C). However, 16% and 9% of the free-flying condors had prothrombin time and Russell's viper venom times, respectively, that exceeded the captive bird mean values by two standard deviations (Fig. 3B), suggestive of anticoagulant exposure and effect in those birds (Hindmarch et al., 2019; Shlosberg and Booth, 2006).

5. Discussion

This is among the first and most widespread assessments of

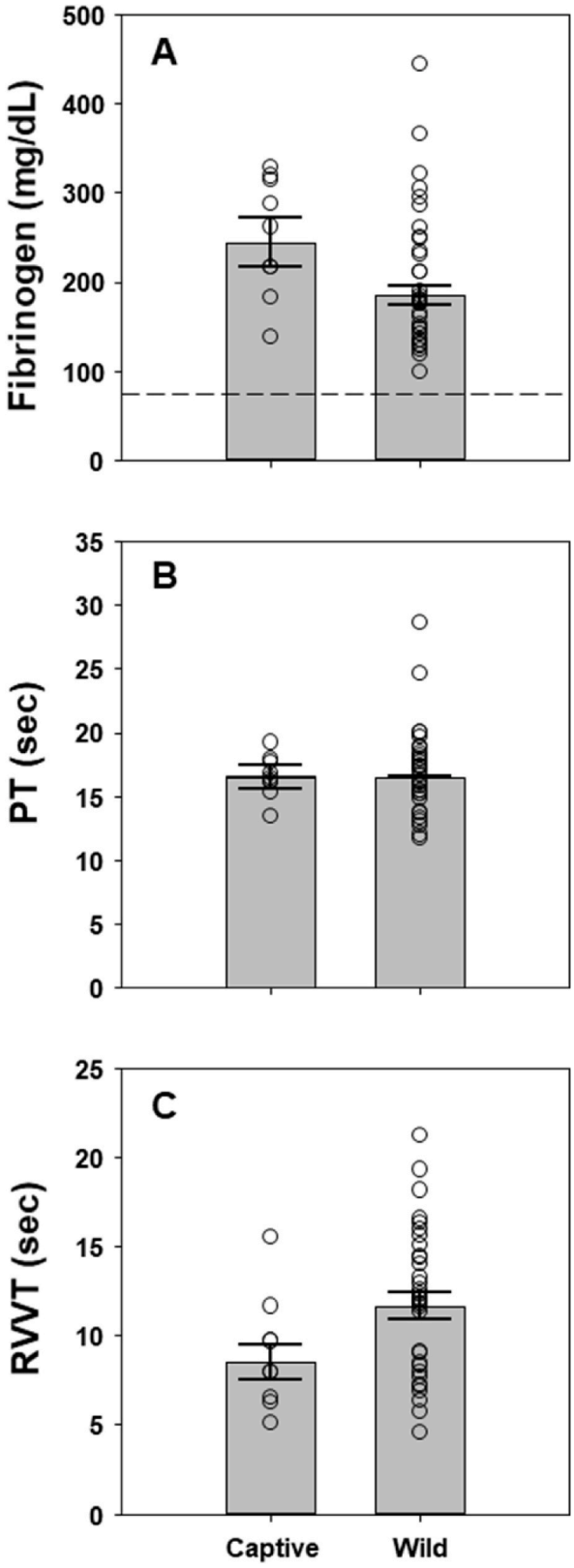

Fig. 3. Fibrinogen concentrations (mg/dL; A), and clotting assay results for prothrombin time (PT; B), and Russell's viper venom (RVVT; C) for captive and free-flying California condors (*Gymnogyps californianus*). Results are model-derived least-squares means ± standard error. Open circles represent individual data points. Dashed line in fibrinogen figure represents the concentration that readily promotes clot formation (~75 mg/dL).

anticoagulant rodenticide exposure in California condors, detailing the incidence rate of exposure as well as some potential factors that influence its probability and residue concentrations in liver. We also found potential evidence of adverse physiological effects associated with AR exposure in free-flying condors. We found wide-ranging exposure in California condors, with residues varying 117-fold, and 25%–50% of birds from all current condor flocks within the United States (central and southern California, Arizona) being exposed. Forty-two percent of the condors and 93% of surrogate vultures contained quantifiable levels of SGARs in their livers. Although concentrations were generally low (<10 ng/g ww), 48% of the exposed California condors and 64% of the exposed turkey vultures exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww; derived from research in other more abundant species of raptors (Thomas et al., 2011). Furthermore, 10% and 13% for condors and vultures, respectively, exceeded the 20% probability of exhibiting signs of toxicosis (>80 ng/g ww). As such, there was some evidence of delayed blood clotting in 16% of the free-flying condors. Notably, we also found that the probability of AR exposure in condors increased with greater SGAR application rates, mediated by precipitation, and liver ΣSGAR concentrations increased across the period of the study. Lastly, initial evidence suggests increased liver ΣSGAR concentrations were also associated with a higher probability of Pb toxicosis as the primary source of condor mortality, indicating that exposure to these two contaminants may be correlated or that there may be an unknown additive or synergistic effect on physiology between the two contaminants.

Different tissues are indicative of a range of AR exposure time frames in birds, with plasma generally reflecting more recent exposure (weeks) and liver residues reflect longer term exposure (up to a year; (Horak et al., 2018; Murray, 2020). We found no evidence of recent AR exposure in condors based on blood plasma concentrations. However, liver samples from deceased condors indicated considerable AR accumulation, either from chronic low-level exposure or from infrequent acute exposures. These findings highlight some of the challenges of monitoring AR exposure in wild birds. Importantly, other indices of exposure may help evaluate the prevalence of AR impairment. For example, we detected that 16% and 9% of the free-flying condors had prothrombin times and Russell's viper venom times respectively outside the upper extreme value of captive condors, possibly indicating recent AR exposure and illustrating the utility of using plasma samples for examining potential physiological effects of ARs. Similarly, we only detected ARs in 10% of the turkey vulture plasma samples, but 93% of their liver samples were positive for ARs. Future efforts to monitor ARs in condors may require a hybrid approach where clotting assays could be used to assess potential recent exposure (Shlosberg and Booth, 2006; Hindmarch et al., 2019) with liver tissue from deceased condors providing a more robust understanding of specific long term AR exposure.

Benchmark probabilities serve as a valuable tool in assessing risk associated with AR exposure when species specific data do not exist (Rattner et al., 2014a; Herring et al., 2017). Although, none of the vultures died from AR toxicosis and only one condor death in this study was directly attributed to AR exposure, California condors and turkey vultures exceeded the 20% probability of exhibiting signs of toxicosis in 10% and 13%, respectively, of liver samples with quantifiable ΣSGAR residues. In the case of the single condor mortality, the ΣAR concentration was 19 ng/g ww, and the ARs detected included the most potent brodifacoum (Erickson and Urban, 2004; Herring et al., 2017). Notably, 48% of the condors that had detectable concentrations of liver ARs exceeded the quantity observed in the condor that succumbed to AR toxicosis. This illustrates well-known inter-individual differences in sensitivity, with many factors affecting onset of toxicosis (Rattner and Harvey, 2021). Although the concentrations associated with the 20% probability of exhibiting signs of toxicosis (80 ng/g ww (Thomas et al., 2011); may be considerably below liver concentrations observed in laboratory studies of birds (e.g., 550–2100 ng/g ww; Newton et al., 1990; Gray et al., 1994; Rattner et al., 2020), they may reflect concentrations associated with the more stressful and challenging experiences of wild birds (Rattner et al., 2020) or differences in toxicity associated with chronic low-level exposure (Rattner and Harvey, 2021). Importantly, the Thomas et al. benchmark probabilities of exhibiting signs of toxicosis need to be used judiciously (Thomas et al., 2011). These benchmarks simply indicate that there is a specific probability that signs of exhibiting toxicosis "may" begin at that concentration, and that we should expect species to vary greatly in their sensitivity to SGARs (Thomas et al., 2011).

Second generation ARs are the most common form of ARs detected in raptors and avian scavengers (Murray, 2011; Thomas et al., 2011; Kelly et al., 2014b; Elliott et al., 2016) because they are more frequently used than FGARs (Murray, 2011: Rattner et al., 2014a; Elliott et al., 2016) and have longer half-lives (Erickson and Urban, 2004; Herring et al., 2017). As such, we only detected SGARs in California condors and turkey vultures, suggesting SGARs may be used ubiquitously throughout the study area. Correspondingly, we found an interactive relationship between precipitation and the AR exposure index (brodifacoum, bromadiolone, difenacoum) within 12-months prior to the condor's death, and the probability that condor livers would contain at least one detectable SGAR. This relationship indicates that the probability of detecting SGARs in condor livers is influenced by SGAR availability, but that relationship is mediated by precipitation. Although the exact mechanisms behind this relationship are not entirely understood, it seems plausible that varying levels of precipitation may regulate small mammal populations (Brown and Heske, 1990; Meserve et al., 2003; Gillespie et al., 2008), potentially influencing the movement of SGARs through condor food webs. Although the specific sources and locations of AR exposure in condors and vultures are unclear, the use of SGARs in the urban/rural interface has been demonstrated as an important exposure source for other taxa (e.g., non-migratory mammalian species (Nogeire et al., 2015; Poessel et al., 2015; Series et al., 2015; Lohr, 2018). Illegal use of ARs in the growing of marijuana is also a possible source (Gabriel et al., 2012; Franklin et al., 2018; Gabriel et al., 2018), in addition to the off label use of ARs by the public purchased through farm supply stores and online sales where sales are not tracked (Quinn et al., 2019). The relationship between legal applications of SGARs and exposure suggest illegal sources of ARs may play a smaller, more localized role in condor exposure to ARs.

Migration and vast foraging ranges of birds like condors can further complicate determinations of the geographic origin of AR exposure. This is particularly the case when measuring exposure using liver because AR concentrations can spike immediately after acute and substantial exposure (Horak et al., 2018) or accumulate over months of chronic low-level exposure (Rattner et al., 2014a; Herring et al., 2017). As a result, AR concentrations in liver may reflect cumulative exposure from areas substantially removed from the sampling location (Kirk and Mossman, 1998). Subsequently, turkey vulture AR exposure could occur locally, on their wintering grounds, or during migration. The monthly home ranges of California condors can span 25–90 km^2 (Rivers et al., 2014) and on average they travel 70 km per day (Hall et al., 2021). Despite this, the probability that deceased condor livers had detectable concentrations of ARs was related to the ΣSGAR cumulative annual application rate and precipitation within the specific regions utilized during the year prior to their death. This suggests that future condor releases and establishment of new managed flocks, such as in northern California, could benefit from assessing the agricultural application rates. Data from surrogate turkey vultures sampled in northern California and southern Oregon suggest that there is a high likelihood that condors will be exposed to ARs; however, legal application of ARs is considerably lower in northern California than compared to the areas of where condors currently exist (California Department of Pesticide Regulation, 2020).

Use of ARs in the state of California are under some of the strictest environmental regulations in the United States (London et al., 2008; Quinn et al., 2019). Yet even with additional regulations applied by the

California Department of Pesticide Regulation in July of 2014 to minimize non-target exposure in wildlife (Quinn et al., 2019), ΣSGAR concentrations in condor liver increased concurrent with increasing legal ΣSGAR application. In fact, geometric mean ΣSGARs increased by 2.5-fold in condor liver since the 2014 pesticide restrictions. Importantly, during that timeframe, SGAR application rates increased 1.2-fold (California Department of Pesticide Regulation, 2020). A new law (Assembly Bill 1788) was enacted in September of 2020 to further reduce use of SGARs in California, and future monitoring will be helpful in determining the effectiveness of this mitigation effort. However, as with the ban of Pb-based ammunition in California for hunting and shooting of pest species in 2019 (Assembly Bill 711), the success of this new law depends on effective enforcement and potential human component of disregarding regulations/laws.

Lead toxicosis is one of the leading causes of death in California condors and is the primary limiting factor for their population recovery (Finkelstein et al., 2012). However, the probability of a condor dying of Pb toxicosis increased with higher ΣSGAR concentrations in liver, although there was some uncertainty around the magnitude of the effect. The mechanisms responsible for this are unclear, but contaminant mixtures can influence an individual's susceptibility to deleterious effects of certain compounds (Heys et al., 2016; Rattner and Harvey, 2021). For instance, binary mixtures of heavy metals have been found to increase mortality rates in sentinel species (e.g., *Daphnia magna*; Le et al., 2013; Vandenbrouck et al., 2009) and have interactive effects on hormone profiles in birds (e.g., common raven *Corvus corax*; Herring et al., 2018). Thus, AR exposure may reduce the overall health of birds such that additional stressors such as Pb poisoning result in their death. Alternatively, AR exposure may merely be correlated with Pb exposure in the wild because consumed mammals such as shot coyotes (*Canis latrans*) and ground squirrels (Spermophilus spp.) potentially contain both AR residues and Pb fragments. Both coyotes and ground squirrels occur in condor diets (Collins et al., 1999; Finkelstein et al., 2020) and are routinely poisoned with ARs (Poessel et al., 2015; Vyas et al., 2017) and shot with Pb-based ammunition (Stauber et al., 2010; Herring et al., 2016). A third explanation for this relationship could be that with increased Pb exposure, condors become anemic due to inhibition of enzymes involved in hematopoiesis (Finkelstein et al., 2012), coincident AR exposure with coagulopathic consequences could result in increased blood loss, exacerbating effects of Pb toxicosis. It is important to note that the causes and outcomes of this interaction between AR exposure and Pb toxicosis are still speculative, but indicate the importance of more definitive studies to elucidate these mechanisms.

6. Conclusions

Management and recovery of California condors is dependent on understanding risks to individuals, flocks, and the overall population. Anticoagulant rodenticide exposure appears to be common in all current condor flocks within the United States and there is potential for exposure in the future free-flying flock in northern California. While the understanding of AR exposure in condors is largely limited to liver tissue residues from deceased condors, continued monitoring of exposure using blood clotting assays is one means of detecting AR exposure in plasma (Hindmarch et al., 2019; Rattner and Harvey, 2021) at a much reduced cost relative to high-performance liquid chromatography-tandem mass spectrometry although the time frame for AR exposure would be very recent (<1 week; Rattner et al., 2014b; Rattner et al., 2020). Plasma samples collected antemortem or postmortem may help confirm suspected cases of AR toxicosis and mortality (Murray, 2020) and improve our understanding of recent AR exposure moving forward with condor recovery efforts. Further elucidating where on the landscape condors are being exposed to ARs would also help in developing plans for mitigate exposure.

Author statement

Garth Herring: Conceptualization, Data curation, Funding acquisition, Methodology, Formal analysis, Project administration, Writing-Original draft preparation. **Collin Eagles-Smith:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing-Original draft preparation. **Rachel Wolstenholme:** Data curation, Funding acquisition, Methodology, Writing- Review and Editing. **Alacia Welch:** Data curation, Funding acquisition, Methodology, Writing- Review and Editing. **Chris West:** Data curation, Methodology, Writing-Review and Editing. **Barnett A. Rattner:** Conceptualization, Data curation, Funding acquisition, Methodology, Writing- Original draft preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Link to data is included in the manuscript after the acknowledgements.

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Data availability—Data can be accessed through Sciencebase.gov at https://doi.org/10.5066/P9LWEXGW and https://doi.org/10.5066/P9NHPLHX.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119925.

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BIRDS

Turkey vultures in California are testing positive for rat poison

Despite statewide restrictions on the deadly poisons, new research shows they're still infiltrating the food web.

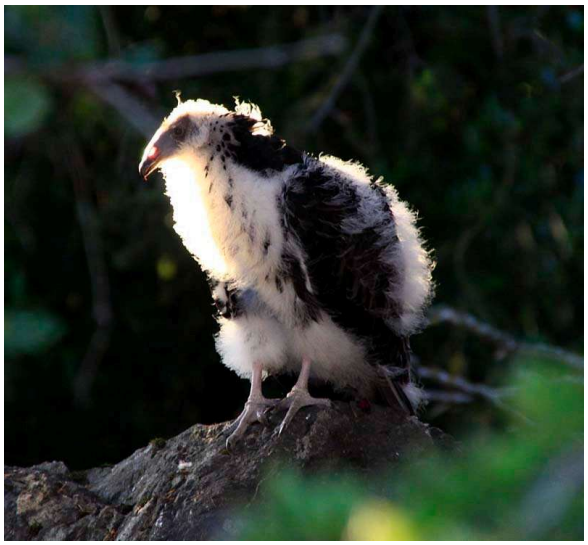
Chi En Kim March 5, 2025

A turkey vulture in Los Angeles, California.
Chon Kit Leong/Alamy

In humankind's ongoing war against rats, rodents are far from the only casualties.

Over the last few years, a **common** class of chemicals known as anticoagulant rodenticides has come under fire for its heavy toll on wildlife. Despite statewide restrictions on these pesticides in California, **a recent study found that** as many as 13% of turkey vultures in the Los Angeles area tested positive for the chemicals. Given the birds' unique ecological perch as nature's carnivorous cleanup crew, the results reveal just how thoroughly anticoagulant rodenticides pervade the ecosystem. They are a reminder of how human actions can have vast environmental consequences, often compounded by climate change — and, in this case, for fundamentally limited returns.

Anticoagulant rodenticides work by causing their victims to bleed to death, often internally. Afflicted animals show signs of anemia and often bleed from their nostrils, mouth and anus before they die. Animal cruelty aside, these substances are problematic because they can persist in carcasses and the environment for **up to a year**. This means that a poisoned rat can in turn poison its predator, and that predator's predator as well, long after the first fatal nibble. The upshot is vast collateral damage: raptors, foxes, **coyotes**, **bobcats** and **mountain lions** — all of which help keep rodent populations in check — have been sickened or killed by these toxins. Occasionally, pets fall victim, too.



A healthy fledgling turkey vulture.
Courtesy of Todd Backman

The same fledgling turkey vulture after it was found sickened by rat poison and collapsed in the patio of a El Cerrito, California, home. ***Courtesy of Patricia Jones***

"I consider them to be like our modern-day DDT, due to the fact that they have infiltrated the entire food web," said Lisa Owens Viani, the director of Raptors are the Solution, a nonprofit that champions wild predators rather than rodenticides as a pest-control solution.

Thanks to the advocacy efforts of groups like **Owens Viani's**, in 2020, California signed into law **a ban** on the most harmful anticoagulant rodenticides by the general public and pest control companies. **In 2023** and **again in 2024**, the state passed additional legislation that added older versions of these rodenticides to that ban.

"I consider them to be like our modern-day DDT, due to the fact that they have infiltrated the entire food web."

Immediately after the first ban was passed, **raptor deaths by poison dropped nearly 15%**, according to data from the California Department of Fish and Wildlife, though the numbers have fluctuated in subsequent years. But the new study, in which tested turkey vultures for the chemicals after the initial bill was enacted, showed that anticoagulant rodenticides still pervade the environment.

According to study author Miguel D. Saggese, an avian and wildlife researcher at Western University of Health Sciences in Pomona, California, the results "provide further evidence that there is still a problem out there for non-target species."

SCAVENGERS LIKE TURKEY VULTURES, with their diverse carrion diet, are good sentinels of rodenticides' footprint across an entire ecosystem. Still, the results might be an underestimate. The new study examined blood samples from live-captured vultures, so the results provide only a snapshot of the birds' most recent encounters with the chemicals. Liver necropsies, which are more telling of chronic exposure, tend to register higher contamination rates — one 2022 study found that **93%** of turkey vultures in Northern California and southern Oregon had anticoagulant rodenticides in their bodies — though necropsy results can skew toward animals that have already perished from the poisons.

Turkey vultures are not a threatened species, but their exposure sounds an alarm for their more vulnerable neighbors. **Spotted owls**, bald eagles and the iconic California condor are already at risk of extinction, and anticoagulant rodenticides are likely a contributing factor. In the past, monitoring efforts have detected the toxins among these birds of prey. The prevalence among turkey vultures indicates that the chemicals need to be eliminated from the environment to ensure the health of wildlife in the West, whether or not the animals are endangered.

A bleeding great horned owl from the Morro Coast at Audubon's Sweet Springs Preserve the day before its death from pesticides.
Courtesy of David Lamkin

A poisoned red-tail hawk that was bleeding right until its death.
Courtesy of WildCare

California is the only state with legislation restricting anticoagulant rodenticides. But even the Golden State's bills have gaping concessions: The agriculture industry and food producers are exempt from the bans, as are public health agencies. And some people still set out illegal rat bait boxes anyway, regardless of what the law says.

Still, there's another compelling reason to renounce anticoagulant rodenticides: They're not all that effective at reining in rats. Experts say that a more durable solution is to not give rodents a reason to come by in the first place — by sealing off food sources and fortifying trash bins. Not only do the relatively slow-acting poisons falter against the prolific reproduction of rodents, they also kill off the rats' natural predators, which are humanity's most valuable allies against rodent infestations. Ultimately, the chemicals we employ to control rat populations end up helping rat populations slide out of control. "None of it makes any sense," Owens Viani said. "I just feel like it's kind of a scam that's been perpetrated to the public."

"I just feel like it's kind of a scam that's been perpetrated to the public."

And climate change is making things worse for pesticides-strained raptors. "Climate change is the very MOTHERSHIP of ecological stressors," wrote Allen Fish, a raptor biologist and former director of the Golden Gate Raptor Observatory, in an email. Already-weakened species may lack the wherewithal to deal with dwindling food sources and shrinking habitats. Meanwhile, warming temperatures allow rats to remain active during mild winters, eating and mating instead of laying low underground, and society's typical response — doling out even more rodenticides — only increases secondary poisoning events.

Anticoagulant rodenticides may well prove the last straw for some species' survival. "It's an ongoing environmental catastrophe that's happening right before our eyes," Owens Viani said.

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RESEARCH ARTICLE

High rates of anticoagulant rodenticide exposure in California Barred Owls are associated with the wildland–urban interface

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ABSTRACT

Pesticide use is pervasive and the exposure of non-target wildlife has been well documented over the past half-century. Among pesticides, anticoagulant rodenticides (AR) have emerged as a particularly important threat in forests of the western United States, with exposure and mortality reported for several species of conservation concern. To further quantify this threat, we collected specimens of Barred Owls (*Strix varia*) and Barred Owl x Spotted Owl hybrids from the Klamath and Cascade Mountains and Sierra Nevada in California, USA to use as indicator species for environmental contamination with AR and to infer exposure of closely related and ecologically similar Northern and California Spotted Owls (*S. occidentalis caurina,* and *S. o. occidentalis,* respectively). We tested 115 Barred Owl and 12 Barred Owl x Spotted Owl hybrid livers for 8 AR compounds and found high rates of exposure (62%) across our study area, and greater than previous studies in the Pacific Northwest. In addition, we sampled 7 ovaries from 7 females and 100% tested positive for AR. Female Barred Owls were more likely than males to be exposed (78% and 50%, respectively). Unlike previous studies, we found no clear link between illegal cannabis cultivation and AR exposure. However, Barred Owls sampled in proximity to the wildland–urban interface (WUI) were more likely to be exposed to AR. Though the exact source (e.g., cannabis cultivation or application around human dwellings) and location are unknown, the association of AR exposure with the WUI was supported from GPS data from Barred Owls, Northern and California Spotted Owls, and hybrids using the WUI for foraging. The high rate of AR exposure in Barred Owls and hybrids provides mounting evidence of an additional stressor that ARs may pose to Spotted Owls—including the first evidence for California Spotted Owls—and fauna native to western forest ecosystems.

Keywords: Barred Owl, brodifacoum, environmental contamination, pesticides, Spotted Owl, *Strix varia*, *Strix occidentalis*, wildland–urban interface

LAY SUMMARY

- Anticoagulant rodenticides have emerged as an important threat in forests of the western United States, and it is vital to understand how and where wildlife is exposed.
- As indicator species for Spotted Owl exposure, we screened 115 Barred Owls and 12 Barred Owl x Spotted Owl hybrids, collected from northern California, USA for 8 anticoagulant rodenticides.
- 62% of owl specimens (72 Barred and 7 hybrid) were exposed to anticoagulant rodenticides, in particular to the acutely toxic, second-generation class.
- Females and owls sampled close to the wildland–urban interface were more likely to be exposed to anticoagulant rodenticides.
- GPS-tagged Barred and Spotted Owls commonly foraged in the wildland–urban interface, suggesting Spotted Owls are also likely at risk of exposure.
- The high rate of AR exposure in Barred Owls and hybrids provides mounting evidence of an additional threat to Spotted Owls.

Las altas tasas de exposición a rodenticidas anticoagulantes en *Strix occidentalis occidentalis* se asocian con la interfaz urbano-silvestre

RESUMEN

El uso de plaguicidas es generalizado y la exposición no deseada de la vida silvestre ha sido bien documentada durante el último medio siglo. Entre los pesticidas, los raticidas anticoagulantes (RA) han surgido como una amenaza particularmente importante en los bosques del oeste de los Estados Unidos, con exposición y mortalidad reportadas para varias especies de interés para la conservación. Para una cuantificación más extensa de esta amenaza, recolectamos especímenes de *Strix varia* y de híbridos de *S. varia* x *S. occidentalis* de las montañas Klamath y Cascade y de la Sierra Nevada en California, EEUU, para usarlas como especies indicadoras de contaminación ambiental con RA y para inferir la exposición de *S. o. caurina* y de *S. o. occidentalis*, dos especies estrechamente relacionados y ecológicamente similares. Evaluamos los hígados de 115 individuos de *Strix varia* y de 12 híbridos de *S. varia* x *S. occidentalis* para 8 componentes de los RA y encontramos altas tasas de exposición (62%) a lo largo del área de estudio, y mayores tasas que la de los estudios previos del noroeste del Pacífico. Además, tomamos muestras de 7 ovarios de 7 hembras y el 100% dio positivo para RA. Las hembras de *S. varia* tuvieron más probabilidad de estar expuestas que los machos (78% y 50%, respectivamente). A diferencia de estudios anteriores, no encontramos un vínculo claro entre el cultivo ilegal de cannabis y la exposición a RA. Sin embargo, los individuos de *S. varia* muestreados en las proximidades de la interfaz urbano-silvestre (IUS) tuvieron más probabilidades de estar expuestos a RA. Aunque se desconoce la fuente (e.g., el cultivo de cannabis o la aplicación alrededor de las viviendas humanas) y la ubicación exacta, la asociación entre la exposición a RA con la IUS se basó en datos de GPS de *S. varia*, *S. o. caurina*, *S. o. occidentalis* e híbridos que utilizan la IUS para buscar alimento. La alta tasa de exposición a RA en *S. varia* y en los híbridos proporciona evidencia creciente de que los RA pueden representar un factor de estrés adicional para *S. occidentalis*—incluyendo la primera evidencia para *S. o. occidentalis*—y la fauna nativa de los ecosistemas forestales del oeste.

Palabras clave: brodifacoum, cannabis, contaminación ambiental, interfaz urbano-silvestre, pesticidas, Strix occidentalis, Strix varia

INTRODUCTION

Pesticide use is pervasive with an estimated 2.5 billion kilograms applied globally each year (Alavanja 2010). The exposure of non-target wildlife to pesticides has been well documented over the past half-century (Grier 1982, Peakall and Kiff 1988), with anticoagulant rodenticide (AR) identified as a particularly widespread and important conservation issue (Stone et al. 1999, Erickson and Urban 2004). Though exposure to AR may result in direct mortality, lesser-understood sub-lethal exposure can also have subtle detrimental effects on non-target wildlife (Riley et al. 2007, Thomas et al. 2011, Serieys et al. 2018). Most accounts of wildlife exposure to AR compounds have occurred in urban or agricultural settings, where the use of rodenticides is frequently permitted for the benefit of human health and mitigation of agricultural damage (Erickson and Urban 2004). However, exposure to AR in remote forest settings is increasingly being reported in the western United States, where multiple species of conservation concern have documented cases of exposure and mortality (Gabriel et al. 2012, 2018, Thompson et al. 2014, Franklin et al. 2018, Wiens et al. 2019). Non-target avian and mammalian predators are particularly vulnerable to secondary AR exposure through the consumption of prey that has ingested rodenticide baits (Stone et al. 1999, Erickson and Urban 2004). Poisoned rodents may be easier prey, because internal hemorrhaging greatly reduces joint mobility, causes lethargy, and reduces escape responses (Brakes and Smith 2005). Mitigating the threat of ARs to non-target wildlife in these forested settings requires understanding which species are exposed, as well as where and how exposure occurs.

Within the past decade, exposure of non-target wildlife to AR has been documented via an unexpected route: illegal cannabis cultivation in remote forests in the western U.S. (hereafter "western forests"; Gabriel et al. 2012, Wengert et al. 2018). Growers use ARs, in addition to other pesticides, to prevent rodent damage to cannabis plants, grow-site infrastructure, and food caches (Gabriel et al. 2012, Thompson et al. 2017). Hundreds of illegal cannabis cultivation sites have been found and eradicated in the foothills and mid-elevation slopes of the southern Sierra Nevada and the Klamath/Cascade Mountains, and an average of 4.5 kg (enough to kill ~22,000 rats from an LD_{50} of 0.27 mg kg^{-1}; Erickson and Urban 2004) of AR are found per site (Wengert et al. 2018). These sites are often located far from other human developments and roads in remote parts of the forests where detection is unlikely (Thompson et al. 2017). However, another source of AR exposure in non-target forest wildlife is from more expected applications around human structures and dwellings located in or near forested settings in what is known as the wildland–urban interface (WUI; Radeloff et al. 2005), defined as where houses meet or are intermixed with undeveloped wildland vegetation. In addition to habitat conversion, exposure of non-target wildlife to ARs is an emerging conservation challenge for wildlife living in close proximity to the WUI (Riley et al. 2007, Serieys et al. 2018).

Whether the exposure is occurring via cannabis cultivation or human communities, exposure to AR in western forests appears to threaten multiple species of conservation concern. For example, high rates of AR exposure have been reported in dead or dying Pacific Fishers (*Pekania pennanti*) in coastal California and the southern Sierra Nevada (85%, n = 101; Gabriel et al. 2012, 2015, Thompson et al. 2014) and in Northern Spotted Owls (*Strix occidentalis caurina*) found dead in coastal California (70%, n = 10; Gabriel et al. 2018). Given the lethal and potential sub-lethal effects of AR, exposure to these pesticides may exacerbate, or even be among the causes of, long-term population declines of both Northern Spotted Owls (Dugger et al. 2016) and California Spotted Owls (*S. o. occidentalis*; Tempel et al. 2013, 2014, Conner et al. 2016) when combined with other key stressors including megafires (Jones et al. 2016), historic habitat loss (Dugger et al. 2016), and competition with invasive species (Long and Wolfe 2019, Wood et al. 2020a). However, given the status of species of conservation concern for both Spotted Owl subspecies, testing Spotted Owls for AR exposure with large sample sizes of liver or blood sampling is difficult and not practical (e.g., obtaining permits).

To characterize Spotted Owls' risk of AR exposure, we used Barred Owls (*S. varia*) as indicator species (Caro and O'doherty 1999) for the presence of AR within the southern Klamath and Cascade Mountains and the Sierra Nevada in northern California. Barred Owls are a closely related and ecologically similar relative of Spotted Owls (Gutiérrez et al. 2007, Wiens et al. 2014) and were first documented within the range of the Northern Spotted Owl in the 1960s (Livezey 2009) and the core range of the California Spotted Owl in the early 2000s (Dark et al. 1998). Barred Owls compete with congeneric Spotted Owls where they occur sympatrically, and there is strong evidence they are one of the causes of declines in Spotted Owl populations (Wiens et al. 2014, Long and Wolfe 2019). Previous work has reported high rates of AR exposure in Barred Owls in Oregon and Washington (48%, n = 40; Wiens et al. 2019), and in coastal California (40%, n = 84; Gabriel et al. 2018). Barred Owls are likely a reasonable, if not conservative, indicator species for AR exposure in Spotted Owls due to a complete overlap in diet and habitat with Barred Owls being less focused on rodent prey than Spotted Owls (Wiens et al. 2014).

In this study, we leveraged biological samples collected as part of an experimental Barred Owl removal study in both the Klamath/Cascades and the Sierra Nevada, which offered a rare opportunity to collect a large sample size at a regional scale. This large sample size allowed us to assess AR exposure across a gradient of conditions likely to influence AR prevalence in the environment, including human density and cannabis cultivation. Furthermore, this is the first study to assess AR exposure in California Spotted Owls through the use of Barred Owls as an indicator species. Because the most useful viable method of testing AR exposure requires the recovery of intact liver tissue from a freshly dead carcass, the collected Barred Owls are a unique opportunity to understand the extent to which both Northern and California Spotted Owls are potentially exposed to ARs within the two sub-regions of our study area. We also GPS-tagged Barred Owls and both Northern and California Spotted Owls to assess the extent to which foraging activities occurred in areas characterized by elevated AR exposure in lethally removed Barred Owls. Finally, we tested the potential of in-utero transfer of AR in *Strix* owls by screening ovaries of AR-positive Barred Owls.

We hypothesized that exposure to AR in forest predators, such as Barred Owls and Barred Owl x Spotted Owl hybrids (hereafter "hybrids"), is influenced by biological factors, such as age and sex, and environmental factors, such as proximity to human communities and the intensity of cannabis cultivation. To test these hypotheses, we quantified the exposure of Barred Owls and hybrids to a suite of AR compounds and evaluated the degree to which exposure was associated with a suite of biological and environmental factors. We predicted higher exposure rates in hybrids, assuming hybrids would have similar foraging behavior to Spotted Owls, which have a dietary niche more focused on rodents than that of Barred Owls (Wiens et al. 2014). We predicted that younger and female Barred Owls would have higher rates of AR exposure as a result of larger dispersal movements (Greenwood 1980). We also predicted that owls exposed to ARs would be in worse physical condition than owls not exposed to ARs, given the potentially deleterious effects of sub-lethal exposure to AR. Among environmental factors, we predicted that Barred Owls collected in areas more likely to be used for cannabis cultivation or closer in distance to either known cultivation sites or the WUI, would have greater exposure to AR. Thus, in addition to characterizing the prevalence of AR in *Strix* owls in two new regions, we aimed to elucidate how behavior and human land use patterns influence AR exposure.

METHODS

Study Area

We collected Barred Owls and hybrids from the southern Klamath and Cascade Mountains and from the Sierra Nevada in northern California (Figure 1) on National Forest lands, national park lands, and private commercial timberlands primarily owned by Sierra Pacific Industries. There was considerable variation in climate, elevation, topography, and vegetation, though both sub-regions were

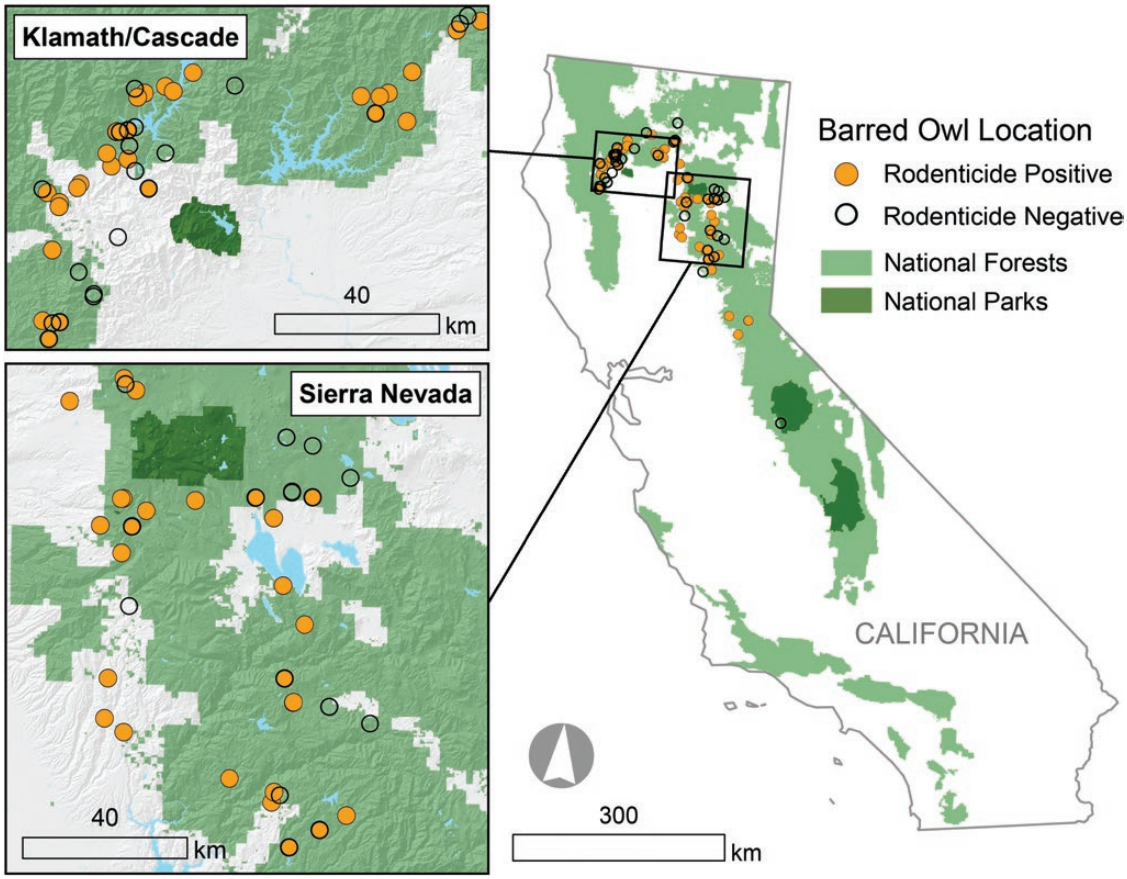

FIGURE 1. Locations of Barred Owls and Barred Owl x Spotted Owl hybrids collected from 2018 and 2019 and screened for anticoagulant rodenticides. Insets at the left show both the Klamath/Cascade and Sierra Nevada sub-regions in California, USA.

predominantly composed of mixed coniferous forest, dominated by ponderosa pine (*Pinus ponderosa*), sugar pine (*P. lambertiana*), incense cedar (*Calocedrus decurrens*), Douglas fir (*Pseudotsuga menziesii*), and white fir (*Abies concolor*). Neither the U.S. Forest Service (S.C. Sawyer, personal communication) nor Sierra Pacific Industries (B.P. Dotters, personal communication) use ARs on lands they manage within our study area. However, there are houses in WUIs adjacent to lands where owls were collected, and it is not known whether ARs are used in these areas.

Tissue Collection and AR Screening

We lured territorial Barred Owls and hybrids by broadcasting digitally recorded Barred Owl vocalizations and collected them with a 12-gauge shotgun following methods described by Diller et al. (2014). We collected Barred Owls and hybrids under federal and state Scientific Collecting Permits (United States Fish and Wildlife Service permits MB24592D-0, MB53229B-0 and California Department of Fish and Wildlife permits SC-002114, SC-11963). We froze owls immediately after collecting them and stored the specimens in a −20°C freezer until we delivered them to the Museum of Vertebrate Zoology (University of California, Berkeley), where we extracted livers and ovaries. We were careful to avoid contamination between the two organs by separating them immediately after they were removed from the abdominal cavity and placing them in separate containers. We thawed all specimens for a similar amount of time to extract tissues, and we left no specimen thawed for over 24 hours. We shipped tissue samples to the California Animal Health and Food Safety Laboratory System (CAHFS; University of California, Davis) where they were screened for 8 commonly used ARs: warfarin, diphacinone, chlorophacinone, coumachlor, brodifacoum, bromadiolone, difethialone, and difenacoum. The first 4 belong within less-acutely toxic first-generation ARs (FGAR); the latter 4 are more acutely toxic second-generation ARs (SGAR) that were created in the 1970s due to rodents developing resistance to first-generation ARs (FGARs; Buckle et al. 1994). High-performance liquid chromatography-tandem mass spectrometry was used to screen tissue samples for AR exposure (whether or not any ARs were detected) and to quantify the concentration of ARs detected (Marek and Koskinen 2007). We classified AR exposure in livers and ovaries using the limit of detection (LOD), which allowed us to detect the presence of

AR in any sample with a concentration above 0.005 μg g^{-1} wet weight (ww). We quantified AR concentrations in liver and ovary samples using the limit of quantification (LOQ), which was 0.050 μg g^{-1} ww for brodifacoum and 0.020 μg g^{-1} ww for all other ARs in owl livers, and 0.200 μg g^{-1} ww for all ARs in owl ovaries (Riley et al. 2007). Any sample above these LOQs could have concentrations quantified. These concentrations all fall below the 0.1 μg g^{-1} ww threshold for mortality rate of 10% of individuals previously reported in Barred Owls (Thomas et al. 2011). When samples had concentrations greater than the LOD and below the LOQ, we designated those individuals as having "trace" exposure.

Calculating Biological Variables

We identified owls in the field as Barred Owls or hybrids based on both plumage and territorial vocalizations. Individuals with vertical barring on the breast feathers and horizontal barring around the nape that produced distinct 2-phrase, 8-note calls (Odom and Mennill 2010) were identified as pure Barred Owls. Individuals with bars and spots on their breast feathers and that produced territorial calls that were not distinctly Spotted Owl or Barred Owl calls were identified as hybrids (Hamer et al. 1994). We classified age as either adult (≥3 yr), sub-adult (1–2 yr), or juvenile (0 yr), based on adults having wider terminal bands than sub-adults on all flight feathers, and juveniles lacking most or all body contour feathers (Mazur and James 2020, J. D. Wiens, personal communication). We determined sex by examining gonads in the lab, and we assessed body condition by characterizing the amount of subcutaneous fat content into four categorical values, with no fat being our baseline ("0"), slight fat ("1"), moderate fat ("2"), and heavy fat ("3"). Because fat reserves in owls change throughout the year (Massemin et al. 1997, DeLong 2006), we obtained a corrected fat index by calculating the residuals of a linear regression of fat against the month of the year (Supplemental Material Figure S1).

Calculating Environmental Variables

We assigned owls that were collected north of the Pit River to the Klamath/Cascade sub-region, and owls sampled south of this river to the Sierra Nevada sub-region (Figure 1). We used this designation to differentiate Barred Owls collected within the range of the Northern Spotted Owl (Klamath/Cascade) or of the California Spotted Owl (Sierra Nevada; Barrowclough et al. 2005). We calculated remaining environmental variables within 2,000 ha circular buffers around collection locations that approximated Barred Owl home range size in the region that we measured using GPS-tagged individuals in a previous study (see Wood et al. 2020a). We used a combination of law enforcement databases (IERC 2019) to calculate the number of known cannabis cultivation sites detected from 2004 to 2019 within the circular buffers. We also related AR exposure to a measure of the probability of illegal cannabis cultivation within the buffers, estimated from a maximum entropy (MaxEnt) model (G. M. Wengert personal communication) parameterized with variables indicative of the suitability of growing cannabis on California's public and private lands. The important variables in this predictive model included elevation, slope, precipitation, canopy cover, stand age, and distances to disturbance, freshwater, roads, and private lands, and used a resolution of 90 m for individual cells. From the MaxEnt model, we obtained an averaged index of cannabis cultivation suitability (ranging from 0 to 1) for each buffer to assess whether owls were more likely to be exposed in areas with more suitable conditions for cannabis cultivation.

Additionally, we calculated the distance of each Barred Owl removal location to the WUI based on 2010 census data (Radeloff et al. 2005, http://silvis.forest.wisc.edu/data/wui-change/), where owls that occurred within the WUI were assigned a distance of 0 km. Both intermix (where housing and vegetation intermingle) and interface (where housing occurs in the vicinity of contiguous wildland vegetation) components of the WUI spatial dataset were used. Four thresholds are defined in the WUI data provided by Radeloff et al. (2005) based on the level of housing density: high, moderate, low, and very low. We chose to use the low density WUI threshold requiring at least 6.17 housing units km^{-2} because of concordance we observed with this threshold and buildings visible in a building footprint spatial layer developed from Microsoft (https://www.microsoft.com/en-us/maps/building-footprints). Finally, we calculated landownership as the proportion of the circular buffers that were composed of National Forest lands. Descriptive statistics of the environmental variables is listed in Supplemental Material Table S1.

Characterizing Barred and Spotted Owl Foraging Activities

To characterize the distribution of Barred Owl foraging locations relative to environmental factors related to AR exposure (in this case WUIs, see below), we GPS-tagged 7 Barred Owls and 3 hybrids between May and August of 2017 and 2018 in the northern Sierra Nevada. We used visual and vocal lures to attract Barred Owls and hybrids and captured them with dho-gaza nets, and applied Argos-enabled GPS backpack tags (Lotek Wireless, Newmarket, Ontario, Canada). We programmed tags to record 4–6 nighttime locations per week between April and August, and then to record 1 location per week between September and March.

We also used locations from 24 GPS-tagged Northern Spotted Owls and 106 California Spotted Owls to characterize their use of areas associated with elevated AR

exposure in Barred Owls—and thus the potential for Northern and California Spotted Owl exposure rates to mirror Barred Owl rates. Northern Spotted Owl locations were collected in the Klamath Mountains between March and August of 2017, and California Spotted Owl locations were collected in the Sierra Nevada between May and August of 2015 through 2020 as part of previous studies (Jones et al. 2016, Atuo et al. 2018, Kramer et al. 2020). We used vocal lures to locate Spotted Owls and captured them either by hand-grab, pan-trap, or snare-poles, and applied GPS backpack tags (Lotek Pinpoint VHF 120, Newmarket, Ontario, Canada). Spotted Owl tags were programmed to record 5 hourly nocturnal locations per night between March and August. From these data, we calculated the mean proportion of locations that occurred within the WUI for both Northern and California Spotted Owls, as well as the proportion of individuals of each subspecies with at least one location in the WUI. We assumed the majority of these locations were primarily foraging locations as owls are nocturnal predators, but we acknowledge that other behaviors such as territory defense, resting, and returns to roosts and nests may be included in these locations.

Additionally, we calculated the proportion of all known Northern Spotted Owl activity centers and all California Spotted Owl activity centers in the Sierra Nevada whose home ranges at least partially overlapped with the WUI to assess the risk of Spotted Owl exposure to ARs via the possibility of foraging in the WUI. We used 2.1 km radius home ranges for Northern Spotted Owls and 1.6 km radius home ranges for California Spotted Owls (Wiens et al. 2014, Blakey et al. 2019). Activity centers were defined as nest locations or geometric centers of daytime roost locations and were obtained from the California Department of Fish and Wildlife (https://www.wildlife.ca.gov/Data/CNDDB/Spotted-Owl-Info). We also used both Northern and California Spotted Owl designated ranges (USFWS 2017) to calculate the proportion of WUI within each Spotted Owl subspecies' range (only including the Sierra Nevada for California Spotted Owls).

Statistical Analysis

We used a set of generalized linear models (McCullagh and Nelder 1989) within an information-theoretic framework (Burnham and Anderson 2002) to test for associations between AR exposure and biological and environmental factors. Because most exposures were at the trace level, we modeled exposure as a binomial response (exposed = 1 and not exposed = 0). Biological factors consisted of species (pure Barred Owl versus hybrid), age, sex, and the index of body condition. Juvenile and un-aged owls were omitted from the generalized linear model because of small sample sizes. Environmental factors consisted of sub-region, proximity to the WUI, number of known cannabis cultivation sites within home ranges, the average index of predictive cultivation for each Barred Owl home range from the MaxEnt model, and landownership.

We used a multi-stage secondary candidate strategy to select top-ranked models (Morin et al. 2020). First, we ran all combinations of biological models and all combinations of environmental models separately. We then identified supported models as those within 5 AIC_c (second-order Akaike Information Criterion corrected for small sample sizes) of the most supported model for each set of models. Second, we combined and evaluated support for variables in the top models from both the biological and environmental sets. In both model-selecting stages, models with uninformative variables (e.g., confidence intervals of variables overlap with zero) were not considered (Leroux 2019). We used the package *MuMIn* in R Studio 1.3.1073 (R Core Development Team 2017) for these analyses.

We also conducted a general Getis Ord-General G high/low cluster analysis (Getis and Ord 1992) to assess the degree to which AR exposure was more clustered than expected at random, less clustered than expected at random, or randomly distributed. We ran separate analyses for owls collected in the Klamath/Cascade sub-region and those collected in the northern Sierra Nevada (where the majority of Sierra Nevada removals were conducted), and only used locations for where owls were exposed, realizing that mates could be non-exposed. To reduce potential biases associated with sampling multiple owls from the same territory, owls collected within 2.52 km (the radius of a 2,000 ha Barred Owl home range in the region; Wood et al. 2020a) of other owls were combined to single points based on the geometric centers of the points. We also conducted a Moran's I spatial autocorrelation analysis with the same condensed points to assess the degree of concordance between different clustering procedures. All spatial analyses were conducted using ArcMap 10.6.1 (ESRI Inc., Redlands, California, USA).

RESULTS

Barred Owl Collections and Liver Analysis

We screened 127 livers (115 Barred Owls and 12 hybrids) for ARs (Figure 1), of which 62% (79 of 127, 72 Barred Owls, and 7 hybrids) tested positive for at least one AR. Brodifacoum and bromadiolone were the only two ARs detected, with 97% (77 of 79) of exposed individuals having exposure to brodifacoum, 15% (12 of 79) to bromadiolone, and 13% (10 of 79) to both. Eighty-seven percent of the AR exposures were at the "trace" level (below quantification limits), with 13% (seven females, and two males) having quantifiable concentrations of AR. Seven of those samples had quantifiable concentrations of brodifacoum

TABLE 1. Generalized linear modeling results from our final stage of model selection used to examine variability in Barred Owls and Barred Owl x Spotted Owl hybrids exposure to anticoagulant rodenticides in northern California in 2018 and 2019. Model covariates include sex and proximity to the wildland–urban interface (WUI). *k* is the number of parameters, and w_i is Akaike's weight. Results for initial modeling steps are provided in Supplemental Material Tables S2 and S3

Model	*k*	ΔAIC_c [a]	w_i
Sex + WUI	3	0.00	0.869
Sex	2	3.95	0.121
WUI	2	9.28	0.008
Intercept only	1	11.94	0.002

[a]Akaike's information criterion corrected for sample size (AIC_c) of top model was 139.5.

(median = 0.084 μg g^{-1} ww, SD = 0.033, min = 0.050, max = 0.150) and three had quantifiable concentrations of bromadiolone (median = 0.150 μg g^{-1} ww, SD = 0.102, min = 0.120, max = 0.310). A total of 7 ovaries were tested for AR contamination and 100% were positive at trace levels (6 contained brodifacoum, 3 contained bromadiolone, and 2 contained both), and all ovaries were from females whose livers also tested positive for the same ARs.

Factors Associated with AR Exposure

After excluding 4 juveniles (because of small sample sizes), 5 un-aged owls, and 2 owls lacking fat scores, 116 individuals (107 Barred Owls and 9 hybrids) were used to conduct the generalized linear model to predict AR exposure. No pairwise combination of variables were highly correlated (all Pearson's *r*'s < 0.6), although distance to WUI and cannabis cultivation suitability were moderately and negatively correlated ($r = -0.42$, $P < 0.01$) – suggesting that cannabis cultivation was more likely to occur near the WUI. The highest ranked model in the biological-only modeling step contained only sex; all other biological variables occurred in models within 5 AIC_c but they were considered uninformative as the 95% confidence intervals overlapped zero and not considered further (Supplemental Material Table S2). The highest ranked model in the environmental-only modeling step contained only distance to WUI; all other environmental variables occurred in models within 5 AIC_c of the top model but they were considered uninformative as the 95% confidence intervals (95% CI) overlapped zero and not considered further (Supplemental Material Table S3). In our second (i.e. combined) modeling step, the top model contained sex and distance to WUI (w_i = 0.869; Table 1). Based on this model, females (78%) were more likely to be exposed to ARs than males (50%; β = –1.448, 95% CI: –2.391 to –0.590; Figure 2). In addition, the probability of AR exposure declined with distance from the WUI (β = –0.146, 95% CI: –0.271 to –0.029) – in other words, Barred Owls sampled near the WUI were more likely to be exposed (Figure 2). Based on this modeling process, there was little support for an association between AR exposure and known grow sites, the predictive index for the suitability of cannabis cultivation, age, species (purebred versus hybrid), body condition, or landownership.

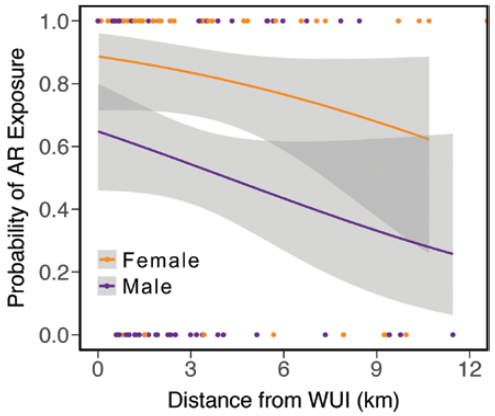

FIGURE 2. Predicted probability of Barred Owls and Barred Owl x Spotted Owl hybrids being exposed to anticoagulant rodenticides (AR) in northern California in 2018 and 2019 plotted against the distance from the wildland–urban interface (WUI; Radeloff et al. 2005). The predicted probability of AR exposure is shown as the solid lines, whereas the 95% confidence intervals are shaded in gray. Colored dots at the top and bottom of the figure represent the raw data of individual owls that were exposed to AR (top) and not exposed to AR (bottom).

We detected little evidence for clustering among locations where Barred Owls were exposed to AR in either the Klamath/Cascade Mountains or the Sierra Nevada. This was the case based on both the Getis Ord-General G high/low cluster analysis (Klamath/Cascade P = 0.27, Sierra Nevada P = 0.83), and the Moran's I analysis (Klamath/Cascade P = 0.39, Sierra Nevada P = 0.58), which indicates that AR was randomly distributed across space in both sub-regions (without considering other environmental variables).

Distribution of GPS-Tagged Owl Locations Relative to the WUI

We tracked the 7 GPS-tagged Barred Owls and 3 hybrids for an average of 229 days (range: 52–392), obtaining an average of 40 foraging locations (range: 15–72) per individual. An average of 2% of Barred Owl and hybrid GPS locations (range: 0–18) occurred within the WUI, and 50% of tagged individuals had at least 1 foraging location within the WUI (Figure 3). We tracked the 24 GPS-tagged Northern Spotted Owls for an average of 65 days (range: 29–79) and obtained an average of 228 foraging locations per individual (range: 94–276). Among Northern Spotted Owls, an average of 2% of GPS locations occurred within the WUI (range: 0–43) and 33% had at least one foraging location within the WUI (Figure 3). We tracked the 106 GPS-tagged California Spotted Owls for an average of 58 days (range: 4–161) and obtained an average of 132 foraging

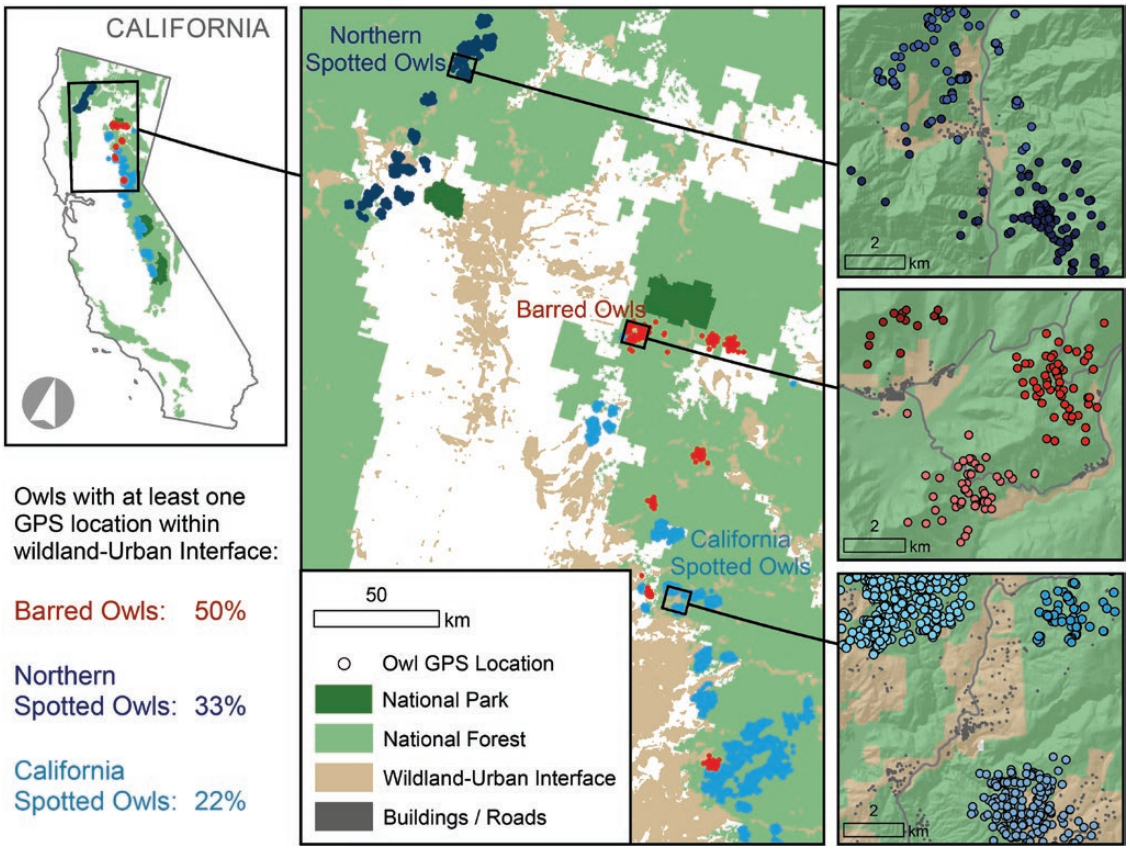

FIGURE 3. Locations and summary statistics of GPS-tagged Barred Owls (n = 10), Northern Spotted Owls (n = 24), and California Spotted Owls (n = 106) in relation to the wildland–urban interface (WUI) in the Klamath/Cascade Mountains and Sierra Nevada in northern California, USA. Dark blue dots on the California map represent GPS-tagged Northern Spotted Owls, red dots represent Barred Owls and hybrids, and light blue dots represent California Spotted Owls. Different color shades in the inset maps represent the GPS locations of individual owls.

locations per individual (range: 9–348). Among California Spotted Owls, an average of 2% of GPS locations occurred within the WUI (range: 0–219) and 22% of tagged individuals had at least one foraging location within the WUI (Figure 3). Based on all known Northern and California Spotted Owl activity centers in the Sierra Nevada, 35% (range: 0.001–1363 ha) and 28% (range: 0.003–751 ha) of individual home ranges overlapped at least partially with the WUI, respectively. However, only 4.3% and 11.9% of Northern and California Spotted Owl ranges overlapped with the WUI, respectively.

DISCUSSION

A high proportion of Barred Owls and hybrids were exposed to AR in both the Klamath/Cascade Mountains and the Sierra Nevada, with exposure being widespread and no evidence for spatial clustering among AR-positive individuals (Figure 1). Females were more likely to be exposed than males and tended to have higher quantifiable concentrations of AR. This is of conservation concern, because we documented, for the first time, AR-positive ovaries and a potential for in-utero transfer of AR in *Strix* owls. AR exposure was not clearly linked to illegal cannabis cultivation, but Barred Owls sampled in proximity to the WUI were more likely to be exposed to ARs. The exposure of such a high proportion of Barred Owls, an apex forest predator, signifies that AR is a pervasive toxicant in western forest ecosystems and contributes to mounting evidence of potential AR exposure in Northern Spotted Owls—and the first potential evidence in California Spotted Owls. Although our sample of hybrids was small, we found similar rates of AR exposure between pure Barred Owls and hybrids, suggesting that Barred Owls may serve as reasonable indicator species for AR environmental contamination and to infer exposure in Spotted Owls. Further support of Barred Owls as reasonable indicator species for Spotted Owl exposure to ARs is provided by the similar use of WUIs by GPS-tagged Barred Owls and Northern and California Spotted Owls. Thus, our study supports previous work showing widespread AR exposure in predators inhabiting remote western forests (Gabriel et al. 2012, 2018, Thompson et al.

2014, Franklin et al. 2018, Wiens et al. 2019), but also suggests that exposure is higher within and around WUIs.

Barred Owl Exposure to AR

Barred Owls collected in our study area were exposed to brodifacoum and bromadiolone. This has important conservation implications because both of these compounds are SGARs and due to the threat they pose to non-target wildlife, their use in California was prohibited in 2014 without a licensed professional, as was their application more than 15 m from human structures (California Code of Regulations Title 3, Section 6471). Indeed, it is unlikely that the high percentage of Barred Owl exposure to AR in our study area comes entirely from legal applications of SGARs, because from 2015 to 2018 only 8.26 kg of brodifacoum were reported to have been sold in the entire state of California (California Department of Pesticide Regulation, https://www.cdpr.ca.gov/docs/mill/nopdsold.htm about the same mass as found at just 2 average illegal cannabis cultivation sites in California (Wengert et al. 2018). Thus, it appears that even with stricter regulations, the legal or more-likely illegal applications of dangerous SGARs and exposure of non-target wildlife remain a challenge for conservation, as does identifying the main sources of illegal applications. Additionally, the proportion of Barred Owls and hybrids exposed to SGARs in our study area (62%) was greater than proportions reported in coastal California (40%, n = 84; Gabriel et al. 2018) and Oregon and Washington (48%, n = 40; Wiens et al. 2019), suggesting that the use of SGARs could be more intense in our study area.

Similar to what has been documented in Oregon and Washington (Wiens et al. 2019), most of our AR-positive specimens had trace liver concentrations below the quantifiable level. As of yet, the sub-lethal effects of ARs and the causes and consequences of trace concentrations in Barred and Spotted Owls have not been studied, although the majority of trace concentrations could be explained by at least 3 non-exclusive possibilities. First, owls with high AR concentrations may have acutely died due to these toxicants and therefore were not available for sampling. If so, our samples may be biased toward the low end of an exposure, with the 9 owls with high concentrations of AR suggesting that concentrations greater than trace levels can occur in Barred Owls. Second, owls may have consumed prey that varied in their concentrations of AR and over different periods of time, which resulted in the majority of, but not all, exposures being at the trace level. However, due to the unknown kinetics of toxicant uptake or sequestration, or degradation mechanisms of AR in *Strix* owls, this possibility will need to be explored further. Third, given that all Barred Owl ovaries tested positive for AR, trace levels could be the result of in-utero transfer of ARs rather than or in addition to the consumption of contaminated prey—a phenomenon that has been reported in Barn Owls (*Tyto alba*; Salim et al. 2015). However, we recognize AR presence in the ovaries still does not necessarily confirm the maternal transfer and that this possibility will need to be explored further by comparing plasmatic vs. ovarian tissue exposure to AR and/or testing eggs directly.

Although the majority of our specimens had trace levels of AR, 9 owls (7 female, 2 male) had concentrations of up to 0.150 μg g^{-1} ww for brodifacoum, and 0.310 μg g^{-1} ww for bromadiolone. These concentrations are both higher than the 0.1 μg g^{-1} ww threshold reported in Barn and Barred Owls, when clinical signs of AR toxicosis begin to show and reflected a mortality rate of 10% of individuals (Thomas et al. 2011). Though not documented in Barred Owls, sub-lethal exposure to SGARs can reduce clutch size and fledgling success in Barn Owls (Salim et al. 2014). In addition, sub-lethal internal hemorrhaging has been documented in Golden Eagles (*Aquila chrysaetos*) and Northern Spotted Owls with liver concentrations of brodifacoum as low as 0.030 and 0.050 μg g^{-1} ww, respectively (Stone et al. 1999, Franklin et al. 2018), and Pacific Fishers have died with signs of AR toxicosis with liver concentrations as low as 0.040 μg g^{-1} ww (Gabriel et al. 2012). More research into the effects of sub-lethal exposure on specific species of concern may be merited, especially because Barred Owl populations are expanding (Wood et al. 2020a) despite high rates of AR exposure. Indeed, no atypical behaviors were observed while collecting Barred Owls who had confirmed trace levels of AR in their tissues. However, the effects of widespread sub-lethal exposure could be more severe in Spotted Owls due to the stress of competitive interactions with more dominant Barred Owls (Wiens et al. 2014), as stress can exacerbate deleterious effects of AR, such as internal hemorrhaging (Cox and Smith 1992).

Biological and Environmental Factors influencing AR Exposure

In contrast to previous studies (Gabriel et al. 2018, Wiens et al. 2019), we found that females were more likely to be exposed to AR than males. Though information is limited for Barred Owls, this may be explained by female Spotted Owls, and female birds in general, having greater dispersal distances on average than those of males (Greenwood 1980, Jenkins et al. 2019). Thus, female Barred Owls, and likely female Spotted Owls, may encounter more sources of ARs that translate to higher rates of exposure and potentially higher concentrations of AR, which also suggests that individuals could have brought AR exposure from natal areas located far from where they were collected. This trend could additionally be explained by Barred Owl females' dependence on males delivering food to them while they are on the nest for a substantial amount of time

every year (Mazur and James 2020). The fact that females had higher rates of exposure is cause for concern because if ovaries testing positive for ARs does indeed signify maternal transfer, it is possible that this transfer is widespread among owls in this study area. However, further research on the possibility of maternal transfer of AR is necessary through the direct testing of eggs.

Higher rates of AR exposure in Barred Owls and hybrids sampled near the WUI indicated that those owls whose home ranges were closer to human development were more likely to be exposed to AR. Indeed, 50% of Barred Owls and hybrids (and 33% of Northern Spotted Owls and 22% of California Spotted Owls) had at least one point in the WUI. Moreover, Barred Owls and hybrids with higher concentrations of AR were collected on average 2 km closer to the WUI than owls with trace AR concentrations, and 3 km closer than owls that were not exposed to AR. However, the mechanism of exposure in the WUI, and whether it is due to cannabis cultivation within the WUI or applications around homes or both, remains unknown. Furthermore, we do not necessarily know where AR-positive owls collected outside of the WUI were exposed. For instance, the half-life for brodifacoum can be as long as 350 days in rats, but predators (including owls) tend to have longer degradation times (up to three times in duration), as demonstrated with the 2–3-day half-life of diphacinone in rats and the 11.7-day half-life of diphacinone in Eastern Screech Owls (*Megascops asio*; Herring et al. 2017). Therefore, it is possible that sampled owls could have been exposed any time over the last 3–4 yr, especially given the apparently recent immigration of some sampled individuals to our study area resulting from vacancies created by removals (D.F. Hofstadter and B.P. Dotters, unpublished data). Nevertheless, we might expect that such discordance between exposure and collection sites resulting from dispersal movements might erode a true association between the WUI and AR exposure, rather than create a false association of WUI and exposure.

Contrary to predictions, AR exposure was unrelated to either of our 2 metrics of illegal cannabis cultivation—an observation that could also have several non-mutually exclusive explanations. First, after California enacted the partial ban on SGARs in 2014, this class of AR was no longer as commonly reported at illegal cannabis cultivation sites, though other toxicants (like FGARs and neurotoxins) were often reported instead (Thompson et al. 2017). Second, illegal cannabis cultivation is by nature clandestine and many grow sites go undetected every year (M. W. Gabriel and G. M. Wengert, personal communication), which could have obscured an actual association to AR exposure. Finally, AR poisoned owls may die near grow sites due to exposure to AR as well as more acutely lethal compounds like neurotoxins, and thus never get sampled. Despite these uncertainties, exposure rates were high in owls sampled several kilometers from the WUI, and particularly so for females—a pattern we consider most likely attributable to either the past or recent use of ARs for illegal cannabis cultivation given low housing densities in these areas (Figure 2).

Threats to Spotted Owls and Western Forest Ecosystems

Our study area adds two new regions to the list of western forests where a high rate of Barred Owls have been exposed to ARs in both remote forested settings and in proximity to the WUI. The 62% of Barred Owls exposed to AR demonstrates that ARs have contaminated the food webs of northern California forests and suggest that AR could pose a threat to wildlife, including Spotted Owls. Although our sample size of hybrids was small, the fact that we did not have any evidence for a difference in exposure rates between pure Barred Owls and hybrids suggests that similar rates of AR exposure could also result in Spotted Owls—a possibility further supported from our GPS foraging locations. In fact, previous work reported 40% (n = 84) of collected Barred Owls and 70% (n = 10) of Northern Spotted Owls that were found dead in coastal California had also been exposed to AR, with Spotted Owls all exposed at trace levels (Gabriel et al. 2018). Spotted Owls prey more selectively on rodents than Barred Owls (Wiens et al. 2014) such that, in regards to diet, Spotted Owls may be more at risk for exposure. However, we found that the proportion of Spotted Owls that frequent the WUI was lower than Barred Owls and also that only a small portion of the WUI overlaps with the U.S. Fish and Wildlife Service designated ranges for both subspecies. Therefore, Spotted Owl behavior and habitat selection may buffer them more from exposure than Barred Owls, which often select suburban habitat containing mature trees (Clement et al. 2019).

In addition to other threats facing Spotted Owl populations, including megafires (Jones et al. 2016), a deficit of large trees (Jones et al. 2018), habitat homogenization (Hobart et al. 2019), and competition with Barred Owls (Wiens et al. 2014, Long and Wolfe 2019), the effects of AR exposure, in comparison, could easily go undetected. Moreover, there is a likely possibility of synergistic effects with sub-lethal effects of AR and other threats faced by Spotted Owls. For example, large disturbances to habitat are correlated to increased cortisol levels in Pacific Fishers (Kordosky 2019) and California Spotted Owl energy expenditure is increased with the presence of Barred Owls in the northern Sierra Nevada (Wood et al. 2020b). Therefore, there is a possibility of environmental stressors accentuating synergistic effects of AR in owls and other forest wildlife.

Our results provide additional evidence that AR exposure could be a more significant threat to forest species of conservation concern than previously thought, and also that it is positively associated with the WUI. This threat is augmented by the long half-life and sub-lethal effects that these toxicants can have (Herring et al. 2017) Exposure in apex predators, like Barred Owls, likely indicates that contamination by AR is pervasive in forest food chains. Indeed, the ubiquity of AR contamination has been documented in many cases, ranging from earthworms and snails being exposed through the soil (Booth and Fisher 2003), to birds eating exposed insects (Masuda et al. 2014), to exposed rodents eaten by various predators, and even to streams, where fish exposed to AR have been reported (Kotthoff et al. 2019). Furthermore, there is the biological significance of low concentrations of AR in various wildlife taxa (Stone et al. 1999, Gabriel et al. 2012, Franklin et al. 2018), suggesting the high rates of trace exposure in Barred Owls and hybrids indicate a significant threat to wildlife, including Spotted Owls.

We believe that future studies should focus on the WUI to elucidate more details on the mechanism of AR exposure, and whether tighter regulations of SGAR applications within the WUI could help to lower this exposure. In fact, as of September 2020, California regulation has recently changed to become stricter regarding the use and application of SGARs (California Assembly Bill No. 1788, Chapter 250). This provides an opportunity to further examine whether further AR exposure is a consequence of legal or illegal applications. Finally, more work is also needed to better understand potential sub-lethal effects and the in-utero transfer of ARs in *Strix* owls, as well as addressing the consequences of high rates of AR exposure in apex predators for forest food webs.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *Ornithological Applications* online.

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Ethics statement: Barred Owl, Spotted Owl, and hybrid handling and tagging procedures were consistent with approved IACUC protocol A005367-R01. Barred Owl and hybrid collection procedures were consistent with approved IACUC protocol A006106-A01.

Conflict of interest statement: The authors declare no conflicts of interest.

Author contributions: MZP, CMW, and MWG conceived the study; MZP and CMW secured funding; DFH, NFK, BPD, KGK, KNR, and CMW contributed to specimen collection; DFH, NFK, and EDF extracted tissue samples; DFH, NFK, CMW, SAW, WJB, BPD, and KNR captured owls and attached GPS tags; MWG and GMW contributed to the cannabis measurements; DFH wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Data availability: Our data is deposited in Dryad. Analyses reported in this article can be reproduced using the data provided by Hofstadter et al. (2021).

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A negative association between bromadiolone exposure and nestling body condition in common kestrels: management implications for vole outbreaks

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Abstract

Background: Vole outbreaks have been extensively described, along with their impacts on humans, particularly in agricultural areas. The use of rodenticides is a common legal practice to minimise crop damage induced by high vole density for biocidal use. However, rodenticides can have negative direct and indirect impacts on non-target species that feed on voles. We studied whether the use of a second-generation anticoagulant rodenticide (SGAR), bromadiolone, can be detected in the blood of fledglings of wild common kestrels Falco tinnunculus in two areas of central Spain, exploring its possible indirect effects.

Results: We found that 16.9% of fledglings had a detectable concentration of bromadiolone in their blood, with an average concentration of 0.248 ± 0.023 ng mL^{-1} . Fledglings with bromadiolone in their blood, regardless of the concentration, had 6.7% lower body mass than those without detectable bromadiolone.

Conclusion: The use of bromadiolone was detectable in the blood of alive non-target species. Detected bromadiolone in blood may reduce the body condition of nestlings, potentially reducing their fitness. The source of bromadiolone found in nestlings needs to be determined in future studies to derive accurate management advice. However, we urge the discontinuation of official SGAR distribution to farmers and their use in agrarian lands to minimise damage of voles on crops, particularly where common kestrels breed, and encourage the use of alternative effective practices. © 2016 Society of Chemical Industry.

Keywords: poisoning; raptors; rodenticide; voles; wild populations.

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PubChem Compound (MeSH Keyword)

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October 24, 2025

RODENT FERTILITY CONTROL AS AN ALTERNATIVE TO POISON

1. GOOD BITES™ RODENT FERTILITY CONTROL

Good Bites™ are palatable rodent-attractive pellets made with nuts, seeds and nutritional grains. The active ingredient is a commercially available supplement, *Tripteryguim wilfordii* Hook F (TwHF).

The Thunder God Vine grows in mountainous regions of China. Extracts made from its leaves and roots have been used for centuries in Traditional Chinese Medicine (TCM) primarily to reduce inflammation (Gao et al 2021).

TwHF active ingredients have rapid onset but because TwHF active ingredients have short half life's, 15 minutes, they do not accumulate in animal tissues and do not pose a risk of non-target exposure (Liu et al 2015). If a predator consumes a mouse or rat even as it leaves a bait station its reproduction will not be effected.

Mice and rats are attracted to the palatable pellets and return to repeatedly feed. Impact on the population is observed in 1-3 months. Due to mice and rat small body mass and their rapid metabolism of TwHF active ingredients there is minimum risk posed to children and adults who would have to eat pounds of pellets daily to begin to achieve an effective dose, practically impossible given the small amounts of pellets available in single feeding stations.

Monitoring of Good Bites™ consumption is essential to its efficient deployment to reach the most mice or rats. Good Bites™ are very appealing to other wildlife such as squirrels, chipmunks, birds and raccoons. We therefore developed a Good Box pellet feeding station to allow only mice or rats to consume Good Bites™.

An additional effect of Thunder God Vine root powder is to induce reversible infertility. In other words, it acts as a ***contraceptive***. This characteristic prevents development of resistance as reported with poisons.

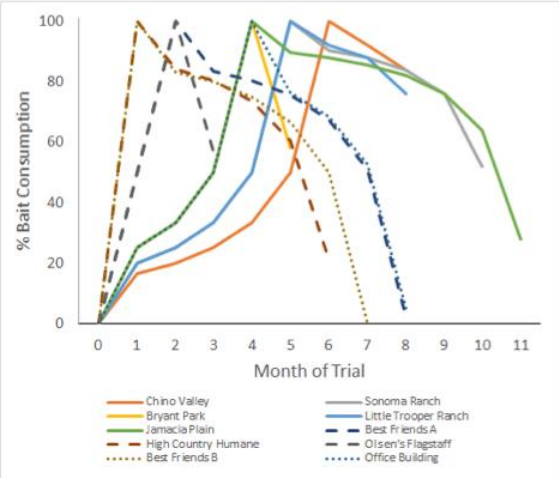
Active ingredients

Good Bites™ (GB) pellets are cereal based and entirely plant derived. They are made of peanut butter, wheat flour, cornmeal, quick oats and table sugar and are highly palatable and attractive to mice and rats. The active ingredient in GB pellets is from Tripterygium wilfordii capsules purchased from Amazon. These capsules contain Tripterygium wilfordii root powder used for hundreds of years in traditional Chinese medicine to provide relief from rheumatoid arthritis inflammation and pain. The active ingredient is triptolide. Triptolide, at ten billionth of a gram or 0.00000001 gram, causes infertility in mice and rats. Triptolide is a contraceptive as the infertility it causes reverses if mice and rats are no longer eating GB pellets. Triptolide is rapidly inactivated by the liver in mice and rats with a half-life of less than 15 minutes. Thus, rodent predators such as raptors are not affected if they catch and eat a mouse or rat that has eaten GB pellets. Triptolide acts on both the rodent ovary and testis, stopping ovulation and sperm development, respectively.

2. PROJECT RESULTS

At present we have conducted over 20 individual studies with 4,321 pounds of pellets deployed and 8,913 data points collected over 2 years from the following settings: retail stores, grain and mill facilities, zoos, sanctuaries, animal shelters, urban neighborhoods reserves and conservation areas. Using scientific published protocols, rodent populations were measured via camera captures, trapping plates, and live captures and correlated to pellet consumption. We developed a web-based application to measure consumption in all locations to determine time to rodent feeding acclimation, population reduction percentages, and sustained reduced populations. There were no adverse effects reported in birds, raccoons, dogs, cats, squirrels, or other small animals. Rodent population reductions because of Good Bites feeding ranged from a low of 10% and high of 99% over a range of 3 to 17 months and sustained for up to 20 months to date.

Consistent among all projects is a pattern (graphed below), wherein consumption climbs to a peak (acclimation) then declines with the reduced rodent population due to reduction in fertility (birth control). Variation in time to decline is dependent upon rodent migration opportunity: Open (parks, ranches, etc.), Semi-Open (buildings, homes), and Closed (interior enclosed buildings)



Pilot Project	Environment	Time to Peak	Reduction	Time to reduction
Chino Valley	Granary - open	6 months	16%	2 months*
Best Friends	Animals – semi-closed	2 months	98%	6 months
Best Friends	Animals - closed	1 month	100%	6 months
Sonoma Ranch	Agriculture - open	5 months	48%	5 months*
Bryant Park	City Park - open	4 months	42 %	1 month *
High Country Humane	Animals – semi-closed	1 month	79%	5 months*
Marco Island	City Park - open	Still climbing	N/A	N/A**
Little Trooper Ranch	Animals - open	5 months	24%	3 months*
Olsen's Flagstaff	Retail – semi-closed	2 months	43%	1 month*
Office Building	Office - closed	4 months	95%	4 months*
Jamacia Plain	Residential - open	4 months	72%	9 months

Summary of all active projects to date:

Category	Project	% Rodent Reduction	Months	Migration
Retail	Flagstaff, AZ	95%	9	Semi-Open
Grain & Mill	Chino Valley, AZ	98%	17	Semi-Open
Urban	Fairfield, CT	53%	7	Open
	Berkeley, CA	10%	5	Open
	Marco Island, FL	48%	12	Open
	Lincoln Park, Chicago, IL	63%	3	Open
	Harlem, NYC, NY	Data collected by NYC staff data 6/26		Open
	Boston, MA	71%	16	Open
	Manhattan, NYC, NY	42%	6	Open
Zoos, Ranches & Sanctuaries	Petaluma, CA	49%	24	Open
	Salt Lake City, UT	39%	3	Semi-Open

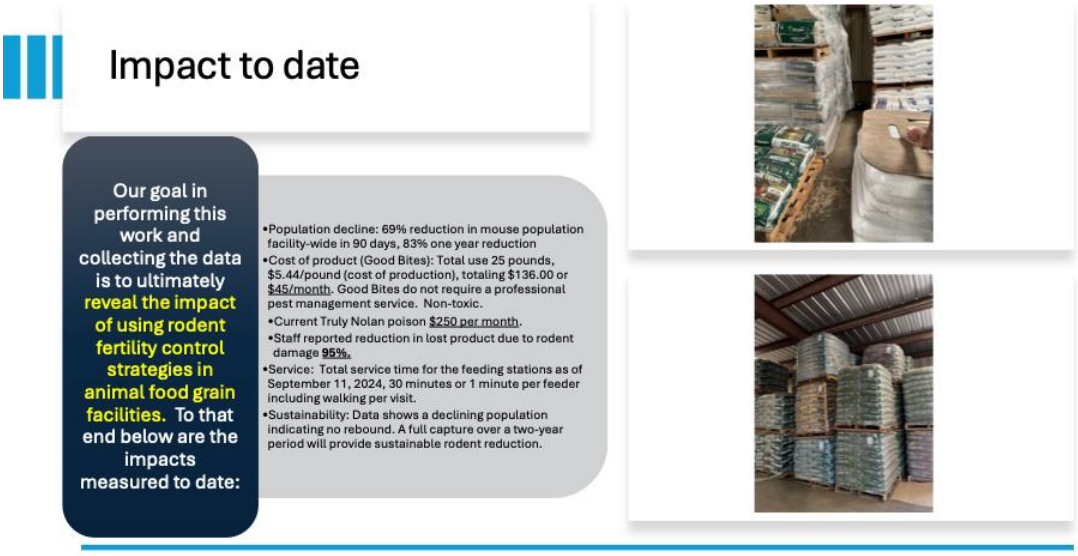
	Kanab, UT	68%	7	Semi-Open
Animal Rescues	Flagstaff, AZ	92%	15	Semi-Open
	Kanab, UT	99%	5	Closed

Website data sets are being loaded in the following formats for all projects: Completion 11/1/25

GRAIN PROCESSING – Olsen's Grain & Mill

- The problem we were asked to address
  - Rodent damage to warehoused feed and grain
  - Rodent damage to milling equipment
  - Reduce Pest Management costs
- Proposed solution
  - Identify problem areas, begin feeding Good Bites, move rodents away from warehousing and assist in mill exclusion and movement
- Execution of the plan
  - Map rodent movements, test feeding stations for best practices
  - Measure feed and grain losses
- Results
  - **95% reduction** in finished product losses

- **Data Collection Detail**
  - Field cameras in use
- Filtered to exclude initial pellet deployment and locate consumption by location
- Feeding stations, EVO Express with Discovery Box inserts, EVO inserts, Good Box
- Initial deployment May 2024
  - Total 1 year population reduction 83%
- Service transferred to Olsen's staff June 1, 2025



Publications to date:

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Other publications on the active ingredient in Good Bites:

TwHF, 900 publications available on PubMed database.

BIOLOGY LETTERS

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Research

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THE ROYAL SOCIETY PUBLISHING

Pathogen biology

Urban rat exposure to anticoagulant rodenticides and zoonotic infection risk

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Anticoagulant rodenticides (ARs) deployed to control rodent pest populations can increase the risk of pathogen infection for some wildlife. However, it is unknown whether ARs also increase infection risk for target rodents, which are common hosts for zoonotic (animal-to-human transmitted) pathogens. In this study, we tested whether rats exposed to ARs were more likely to be infected with zoonotic pathogens, specifically *Leptospira* spp. or *Escherichia coli*, after controlling for known predictors of infection (i.e. sex, age, body condition). We collected biological samples from 99 rats trapped in Chicago alleys and tested these for *Leptospira* infection, *E. coli* shedding and AR exposure. We found that rats that had been exposed to ARs and survived until the time of trapping, as well as older rats, were significantly more likely to be infected with *Leptospira* spp. than other rats. We found no significant association between *E. coli* shedding and any predictors. Our results show that human actions to manage rats can affect rat disease ecology and public health risks in unintended ways, and more broadly, contribute to a growing awareness of bidirectional relationships between humans and natural systems in cities.

1. Introduction

Anticoagulant rodenticides (ARs) are one of the most common types of substance used to control rodent pest populations; however, little is known about potential unintended, *sublethal* AR effects on rodents. In other species, AR exposure has been associated with numerous sublethal effects (in addition to acute toxicity). For example, sublethal AR exposure can increase infection risk in urban predators (e.g. bobcats, *Lynx rufus*; mountain lions, *Puma concolor*; coyotes, *Canis latrans*; [1–3]) and has been linked to higher parasite and pathogen burdens in birds (e.g. great bustards, *Otis tarda*; [4]). Wildlife exposed to ARs may be more susceptible to infection because ARs have been shown to disrupt immune function [5]. Like the species above, rodents might also experience greater infection owing to AR exposure; in turn, this is relevant to human health as rodents are common hosts for zoonotic pathogens [6–8], especially in human-dominated areas [9]. ARs do not kill immediately; first-generation ARs require multiple feedings to provide a lethal dose, and second-generation ARs—more potent compounds that can kill after a single dose—typically lead to death in 5–10 days [10]. If infection risk is heightened during the period between AR exposure and death, widespread AR use might increase population transmission of pathogens among rodents. Additionally, this could pose a risk of zoonotic pathogen transmission.

Understanding any unintended effects of rodent control on rodent disease dynamics is important because commensal rats carry dozens of zoonotic pathogens [11,12], come in close proximity to people [13], and have a near-global distribution [14]. Brown rats (*Rattus norvegicus*) and black rats (*R. rattus*) can

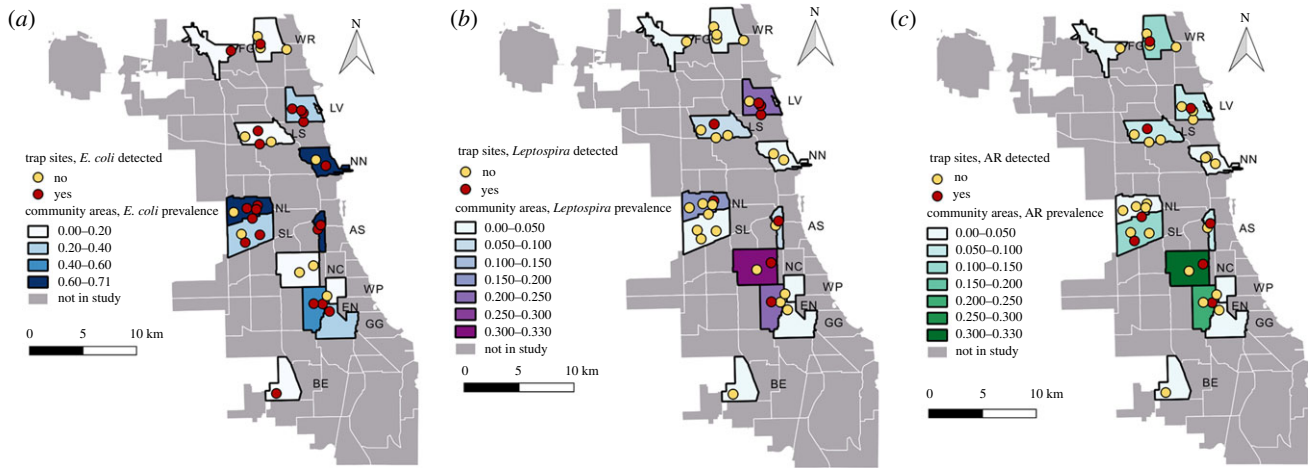

Figure 1. Maps of study community areas (polygons) and trap sites (circles) in Chicago. Colours show the prevalence (shading) or the presence (darker circles) of rats with (*a*) *E. coli* shedding, (*b*) *Leptospira* spp. infection, and (*c*) anticoagulant rodenticide (AR) exposure. Abbreviations correspond to table 1.

carry several environmentally transmitted pathogens that cause human disease (e.g. *Leptospira interrogans*, pathogenic *Escherichia coli*; [15]). Leptospirosis in particular poses a large public health burden, causing an estimated 434 000–1 750 000 cases and 23 800–95 900 deaths in humans annually [16]. Among major cities in the USA, *Leptospira* seroprevalence in rats ranges from 44.1 to 65.3% [17]. Environmental features and management practices can modulate *Leptospira* prevalence. For example, in Chicago, IL, rats trapped in high-income areas with more standing water complaints were more likely to be infected with *Leptospira* spp. [18], while in Vancouver, Canada, rodent control via rat trapping was associated with higher *Leptospira* prevalence [19]. Importantly, low-income urban residents can be disproportionately exposed to rat-associated zoonoses [20] and lower-income countries are often reliant on ARs for rodent control [21]. It is thus crucial to understand how other widespread management practices such as use of ARs could also influence infection dynamics in rats.

In this study, we tested if rats exposed to ARs were more likely to be infected with zoonotic pathogens, specifically *Leptospira* spp. or *E. coli*, after controlling for known physiological predictors of infection. We focused on these pathogens because they are zoonotic, transmitted through the environment, and present in our study population [18]. Based on previous work in urban carnivores, we predicted the probability of *Leptospira* spp. infection and *E. coli* shedding would be higher for rats with detectable concentrations of common ARs in liver tissue relative to other rats. We also predicted the probability of *Leptospira* spp. infection and *E. coli* shedding would be higher for rats that were female, older, and in poorer body condition because these biological factors are known predictors of infection [18,22–24]. Our results will help design best practices for rodent management to protect public health and advance our understanding of how pest management affects urban wildlife ecology.

2. Methods

As part of a previous study [18], 254 rats were trapped in 13 community areas in Chicago, a city with numerous rat complaints (figure 1). Trapped rats were measured, examined for injuries, weighed, and sexed. Rats were considered to be brown rats based on ear and tail morphology, but this assumption was not verified with genetic analyses. A subset of 202 rats were necropsied and screened for environmentally-transmitted bacterial pathogens [18]. Rat kidney tissue was tested for *Leptospira* spp. using polymerase chain reaction (PCR) and rat colon contents (i.e. faeces) were tested for *E. coli* using aerobic culture [18] at Wyoming State Veterinary Laboratory. From these rats, we selected 99 (table 1) to be screened for seven commonly used ARs (first-generation: chlorophacinone, coumachlor, diphacinone, warfarin; second-generation: brodifacoum, bromadiolone, difethialone). Rats were chosen for screening such that sample sizes would be roughly balanced by capture location, sex, age and infection status. Liver screening was performed by the Animal Disease Diagnostic Laboratory at Purdue University (West Lafayette, IN) using high performance liquid chromatography. Method detection limits (lowest concentration that can be confidently identified) for each AR in liver tissue were as follows: chlorophacinone and diphacinone: 0.25 ppm; coumachlor and warfarin: 0.5 ppm; brodifacoum, bromadiolone and difethialone: 1.00 ppm. Animal use was deemed exempt from Lincoln Park Zoological Society IACUC approval because rat samples were procured through pest management professionals (protocol number 2019–005).

We used generalized linear mixed models (GLMMs; binomial distribution, logit link) to test whether infection status varied by rodenticide exposure status (binary; we considered a rat exposed to poison if at least one AR was detected in the liver) as well as other biological predictors previously found to influence rat infection status. We constructed two GLMMs, one with a response variable of *Leptospira* infection status (positive or negative) and the other with a response variable of *E. coli* shedding status (positive or negative). Explanatory variables for each model included AR exposure status, sex, age class and body condition. We estimated rat age in days based on their mass using growth curve equations, following the methods of [25], and binned rats as younger (30–65 days) or older (greater than 65 days; electronic supplementary material, dataset). We quantified body condition using the scaled mass index [26] using tip-to-tip length (i.e. tip of nose to tip of tail) because it was most highly correlated with mass (see the electronic supplementary material for more detail). While injuries have also been found to be associated with infection [15], we did not include this as a variable because we observed only a few, mild wounds in the study population. Given the low sample size, only main effects of the explanatory variables were considered. We also included capture location (i.e. community area) as a random effect to account for non-independence among samples from the same neighbourhood. Analyses were performed using the glmmTMB package [27] in the R statistical environment v. 4.0.3 [28].

Table 1. Sex, age class and anticoagulant rodenticide poisoning status of rats, separated by trapping location (community area).

	sex		age class		poisoning status	
community area	F	M	younger (30–65 days)	older (>65 days)	AR detected	AR not detected
Armour Square (AS)	5	10	14	1	1	14
Beverly (BE)	1	0	1	0	0	1
Edge Water (ED)	1	1	1	1	1	1
Englewood (EN)	0	4	3	1	1	3
Forest Glen (FG)	1	0	1	0	0	1
Greater Grand Crossing (GG)	2	2	2	2	0	4
Lake View (LV)	14	6	16	4	2	18
Logan Square (LS)	11	5	14	2	1	15
Near North Side (NN)	5	2	7	0	0	7
New City (NC)	2	1	1	2	1	2
North Lawndale (NL)	4	1	4	1	0	5
South Lawndale (SL)	11	2	8	5	2	11
Washington Park (WP)	0	1	1	0	0	1
West Ridge (WR)	7	0	6	1	1	6

3. Results and discussion

We analysed infection status as a function of AR exposure, sex and age class for 99 rats that were trapped in 14 community areas (table 1). Ten liver samples were positive for AR residues (6 females, 4 males; 2 older, 8 younger). Specifically, seven were positive for second-generation ARs (brodifacoum: $n = 3$, bromadiolone: $n = 3$, difethialone: $n = 1$) and three were positive for first-generation ARs (diphacinone: $n = 3$). *Leptospira* prevalence was higher for AR-exposed rats (30%, 3/10) than for unexposed rats (7.9%, 7/89), and *E. coli* prevalence was higher for AR-exposed rats (50%, 5/10) than for unexposed rats (42%, 37/89; figure 2).

GLMMs indicated that AR exposure status was a significant predictor of *Leptospira* infection status (odds ratio = 7.02, 95% CI = 1.10–45.0, $p = 0.04$), as was age class (figure 2 and electronic supplementary material, table S1). Older rats (greater than 65 days) were significantly more likely to be infected with *Leptospira* spp. than younger rats (30–65 days; odds ratio = 5.88, 95% CI = 1.20–28.9, $p = 0.03$). Neither sex nor SMI was a significant predictor in the model. The marginal R^2 (i.e. proportion of variance explained by fixed effects) for the *Leptospira* infection model was 0.21, while the conditional R^2 (i.e. proportion of variance explained by both fixed and random effects) was 0.33. No explanatory variables were significant predictors of *E. coli* shedding status. The marginal R^2 for this model was 0.01, while the conditional R^2 was 0.12.

We found that rats exposed to ARs that survived until the time of trapping were significantly more likely to be infected with *Leptospira* spp. than other rats. Though it is known that ARs can promote infection risk in non-target wildlife, our results demonstrate increased zoonotic infection risk in target rodents. This result is significant for public health and urban ecology because commensal rodents are abundant reservoirs of zoonotic pathogens in cities. More generally, this relationship between rodenticide exposure and infection risk demonstrates an unintended effect of wildlife management on a target species that can feed back to human health.

AR-exposed rats may be more susceptible to infection in the period between exposure and death because of immunomodulatory effects of ARs. Rats exposed to warfarin for 30 days exhibit increased lymphocytes, basophils and monocytes [29,30], suggesting immune dysfunction. In carnivores, AR exposure has been associated with immune dysfunction consistent with cytokine-mediated inflammatory processes, including the suppression of neutrophils [31]. These phenotypic changes might interfere with rodents' ability to mount an effective defence when exposed to infectious leptospires in the environment. Although we quantified rat exposure to rat poison as a binary status, the detection limit in our study exceeded concentrations deemed indicative of acute AR poisoning in other species (200 ng g^{-1} or 0.2 ppm; [4]), suggesting they were high enough to interfere with physiological processes. If rats are more likely to become infected with *Leptospira* spp. after consuming ARs, infection would have to occur before the poison kills the rat (approx. 1 week). Experimental work has demonstrated successful *Leptospira* infection 7 days post-infection [32,33], yet further work is needed to examine *Leptospira* spp. infection dynamics at a shorter timescale and determine how long rats can survive following AR exposure.

Alternatively, infected rats might be more likely to consume poisoned bait. For instance, infected rats could be more attracted to bait stations if they have less energy to actively forage for other food. However, rats are considered asymptomatic, chronic carriers of *Leptospira* ([17]; though see [34]), suggesting it is unlikely that infected rats are more likely to consume AR bait. Future work could also investigate behavioural and physiological changes in poisoned and infected rats to clarify causal mechanisms.

Interestingly, the only other study, to our knowledge, to examine AR poisoning and infection risk in target rodents found that common voles (*Microtus arvalis*) infected with

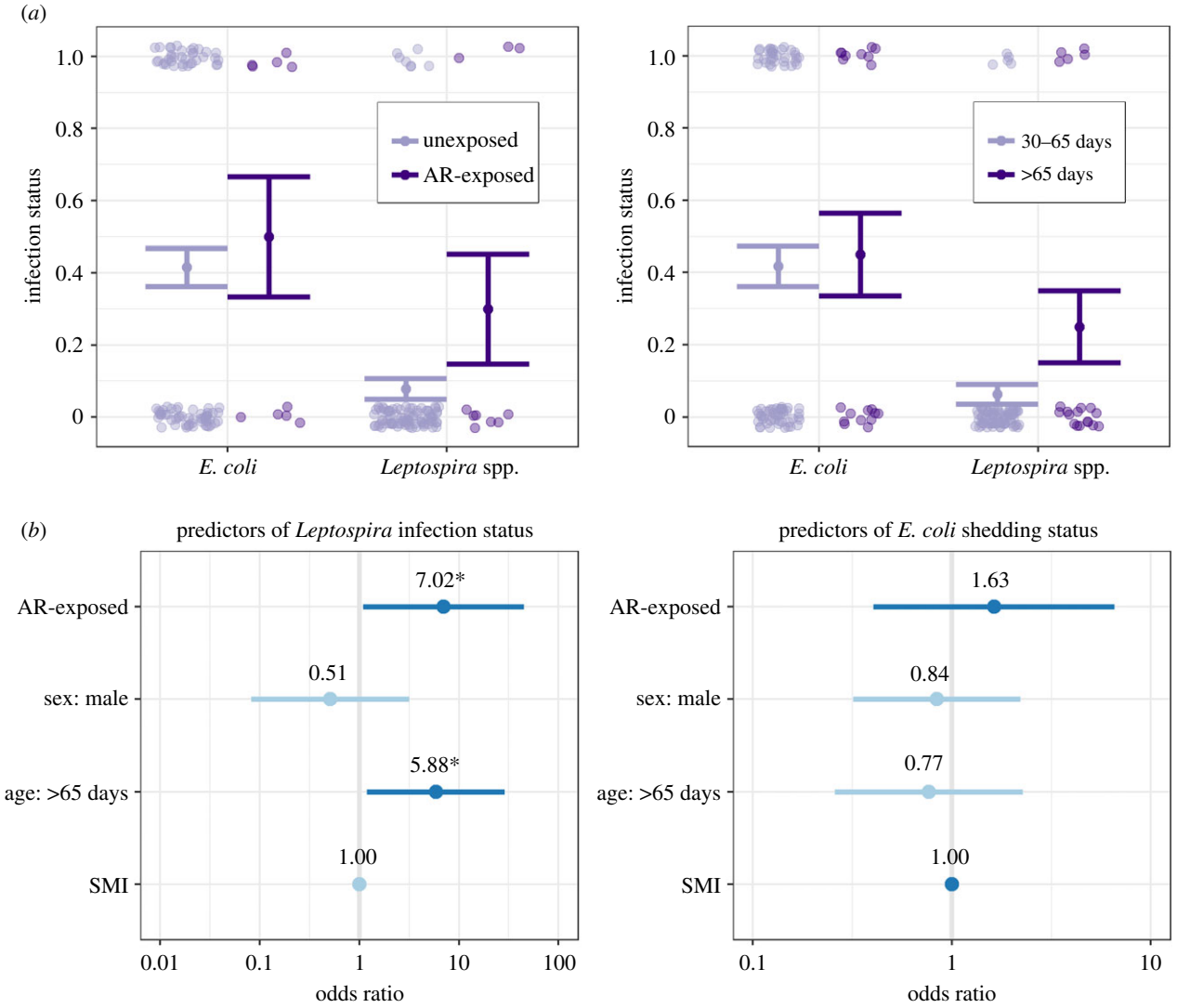

Figure 2. (*a*) Pale-shaded points display binary infection status and solid points and lines represent means and standard errors of infection prevalence. (*b*) Points and lines represent odds ratios and 95% confidence intervals for predictors of infection status from GLMMs. Darker blue lines indicate odds ratios greater than 1, while lighter blue lines indicate odds ratios less than 1. 95% confidence intervals that cross the vertical line at 1 indicate that a predictor is not significant. Asterisks indicate $p < 0.05$.

Francisella tularensis had lower concentrations of the AR chlorophacinone relative to uninfected voles [35]. These results likely differ from ours because all poisoned voles were found dead rather than trapped and *F. tularensis* infection is fatal in voles. However, these differences highlight the need to understand interactions among ARs, pathogens, and hosts with different ecologies. Future epidemiological surveys and experimental work could help identify which types of pathogenic infections are affected by AR exposure.

We also found that older rats were significantly more likely to be infected with *Leptospira* spp. than younger rats. This aligns with previous research and is likely attributable to a greater chance of exposure and infection over time [22]. We might not have found significant associations with other biological factors because of small sample size, which could also explain the relatively large confidence intervals around the odds ratios (figure 2). Contrary to our predictions, we found no association between AR exposure and *E. coli* infection. We may not have detected an increased risk of *E. coli* infection in poisoned rats because our methods could only detect active shedding of *E. coli* in faeces, rather than true infection. Although this is informative for public health, rats could have been infected with *E. coli* but not actively shedding, which might have confounded our results. In addition, while we accounted for non-independence among rats within the same community area using a random effect (under the assumption that community areas are statistically independent from one another, supported by the small home ranges of rats (less than 200 m) [36]), our results may have been confounded by spatial autocorrelation.

Our results add to a growing literature showing environmental hazards of managing rats using ARs, and highlight potential unintended and unpredicted effects of AR exposure on the ecology of rat-associated pathogens of public health importance. Apart from disease ecology, urban rats have exhibited genetic resistance to ARs for decades. Resistant rats carry genetic mutations in the *Vkorc1* gene that interfere with anticoagulant effects on blood clotting [37], rendering the rats less susceptible to anticoagulants. Rats have exhibited genetic resistance even as new generations of ARs are developed [38,39], demonstrating how lethal management can have evolutionary consequences for zoonotic hosts [40]. AR resistance may have important consequences for leptospiral shedding if ARs act as modulators of immune and inflammatory responses and resistant rats are less likely to die

following AR exposure. Instead of relying on ARs, integrated pest management might offer a more sustainable approach by improving urban sanitation and rodent exclusion [41]. Such an approach would align with One Health principles and prevent mortality of urban carnivores, which provide ecosystem services such as rodent population control. More broadly, our results contribute to a growing awareness of bidirectional relationships between humans and natural systems in cities: in our case, that human actions to manage rats can affect rat disease ecology and public health risks in unintended ways.

Ethics. Animal use was deemed exempt from Lincoln Park Zoological Society IACUC approval because rat samples were procured through pest management professionals (protocol no. 2019-005).

Data accessibility. The dataset used in our analysis is available on Zenodo at https://zenodo.org/badge/latestdoi/387547164 [42].

Authors' contributions. M.H.M. led the conceptualization of the study and the collection of biological samples. C.A.S. contributed to project design and led the statistical analysis. M.H.M. and C.A.S. wrote and edited the manuscript, approved the final version of the manuscript agree to be held accountable for the content therein.

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

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GROWTH PERFORMANCE OF NESTLING BARN OWLS, *Tyto Alba javanica* IN RAT BAITING AREA IN MALAYSIA

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ABSTRACT

The growth of nestling barn owls, *Tyto Alba javanica* in immature oil palm in Malaysia was investigated under rat baiting with three different rodenticides. Four treatment plots were established with three plots baited each with warfarin, brodifacoum and a protozoan based biorodenticide, *Sarcocystis singaporensis* plus a fourth non-baited control plot. Three rat baiting campaign were carried out during the study, the first rat baiting campaign was conducted in October 2008, the second was in March 2009 (except for biorodenticide baiting was conducted a month earlier), and the last third baiting campaign in October 2009. The baiting campaigns coincided with the breeding season of barn owl. Nestlings body measurements namely: body mass, culmen length, tarsus length, wing length and tail length were taken after the third baiting campaign, from September 2009 to January 2010. Measurements were recorded every three days from hatching up until 49 days old, i.e., several days before fledging. Nestlings in control plot showed superior for all parameter taken compared to rodenticides treated plots. Body mass of nestlings in control plot were heavier by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The culmen and tarsus length of nestling barn owls reached the adult size during the growth period; while culmen length in control plot was longer by 3.07%, 5.28%, and 1.41% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The tarsus length of nestlings in control plot was also longer by 2.40%, 3.08% and 3.36% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. In contrast with culmen and tarsus length, wing and tail length still grew until day 49 i.e., several days before fledging. The wing and tail length in control plot was shorter by 15.77% and 13.73% compared to adult size. Teratogenic sign was shown by one nestling in brodifacoum treated plot, where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Wing and tail length in brodifacoum treated plot was shorter by 15.26% and 18.24%, respectively compared to control plot.

Keywords: *Tyto Alba javanica,* growth performance, nestling, warfarin, brodifacoum, *Sarcocystis singaporensi,* teratogenic sign.

INTRODUCTION

The barn owl, *Tyto Alba* is widely distributed around the world, occurring in all continents in a wide range of habitats, except Antarctica and the smaller Pacific Islands (Smith and Cole, 1989). There are 36 subspecies including *T. Alba javanica*, found in Peninsular Malaysia and also in Sumatra and Java Islands of Indonesia (Taylor, 1994). Due to its wide distribution, the barn owl has been extensively studied (Bunn *et al*., 1982; Newton *et al.*, 1991; Eason *et al.*, 2002). In Malaysia, *T. Alba javanica* is commonly propagated to control rats in plantation (Lenton, 1984; Smal, 1989; Hafidzi *et al*., 1999). Since 1970's, its distribution had rapidly expanded and the status changed from rare in the late 1960s to common (Duckett, 1984; Duckett and Karrupiah, 1990). The increase of *T. Alba javanica* population in the Peninsular Malaysia was associated with the phenomenal increase in oil palm acreage. This brought about rat outbreaks which translate into readily available food source. Previously, when rat damage reaches threshold levels planters usually resort to warfarin, the first generation anticoagulant rodenticide to deal with the infestation (Duckett, 1984). However prolonged exposure to warfarin, triggers resistance to the latter, prompting more planters to switch to brodifacoum, a second generation anticoagulant rodenticide introduced in the early 1980s. The use of brodifacoum has caused marked decline in rat populations in oil palm (Duckett, 1984; Wood and Fee, 2003). The downside of bodifacoum is it was proven toxic to *T. Alba* from field observations and laboratory studies. The potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to barn owl as non-target animal by direct consumption or secondary poisoning from build-up of rodenticide residues (Newton *et al*., 1990; Shore *et al*., 1999; Dowding *et al*., 2010). Based on that fact, several workers try to find safer rodenticide to replace brodifacoum with an equally effective but have less impact on non target organism. One such alternative is the biorodenticide based on *Sarcocystis singaporensis*, a protozoan pathogen that has been proven effective against rat in the rice field but does not causes harm to humans other animals, such as fish, other mammals and birds apart form rats of the genus *Bandicota* and *Rattus* (Jakel *et al*., 1996; Jakel *et al*., 2006). Although there have been many studies on the effects of chemical rodenticides on adult barn owl, very little information exist about the effects of chemical rodenticides on growth and development of nestlings. Therefore the objective of this study is to evaluate the effects of regular rat baiting on the growth performance of the nestlings barn owl, *T. Alba javanica* in an immature oil palm area in Malaysia.

MATERIALS AND METHODS

Location and period of study

The study was conducted in immature oil palm at FELCRA oil palm plantation scheme in Seberang Perak ($4^0$02´N, $100^0$53´E), Perak, Malaysia from September 2008 to January 2010. The study sites constitute part of the replanting area started in mid 2007.

Rat baiting and treatment

Twenty two artificial nest boxes, made of wood, were set up in April to June 2008 in the immature palm by Felcra management. Four treatment plots were established. The area for each plot is no less than 100 ha. Three plots were baited with warfarin, brodifacoum and the biorodenticide *Sarcocystis singaporensis*, respectively. The fourth was left untreated and served as the rodenticide-free control plot. The average nest box density was 1 box for 25 ± 3.83 ha. The first baiting campaign for all three rodenticides was carried out on 20-25th October 2008. The second baiting campaign for warfarin and brodifacoum on 10-12th March 2009, while second baiting campaign for biorodenticide was carried out on 25-27th January 2009. Third baiting campaign was carried out on 28 September to 3rd October 2009 for all three rodenticides. The baits were placed at the base of the palm tree. In the first campaign, a single round of baiting was carried out while two baiting rounds were conducted in the second and the third baiting campaign.

Data collection

48 nestlings were selected for this study: 14 from control plot, 12 from warfarin treated plot, 9 from brodifacoum treated plot and 13 from biorodenticide treated plot. They were weighed at three day interval for up to 49 days i.e., several days before fledging. The day of hatching was designated as day zero (Janiga, 1992) for monitoring growth rate. Hatched siblings were individually marked using different colored ribbons tied to the leg for age determination during later stages of growth. All observations were carried out in the nests from 5 to 7 p.m during the breeding season, i.e., from September 2009 to January 2009.

Nestling growth metrics

For nestling growth metrics, five measurements were taken, namely: body mass, culmen length, tarsus length, wing length and tail length. Body mass was measured using Apex A-5001, a portable digital weighing scale (accurate to 1 g); culmen length was measured using Mitutoyo Caliper, from the tip of the upper mandible to the base of the culmen, to the nearest 1 mm. Tarsus length was measured from the top of the tarsus (just below the tibio-tarsal joint) to the joint at the base of the middle toe, to the nearest 1 mm. Tail length was measured from the fold of skin between the central tail-feathers and the tip of the longest tail-feathers, to the nearest 1 cm. Wing length was measured from the bend of the folded wing to the tip of the longest primaries (Weick, 1980; Janiga, 1992).

Statistical analysis

Data of similar-aged nestlings from all the nest boxes were pooled to calculate the mean for different growth and to analyze the pattern of growth changes in the measured variables using Kruskal-Wallis test. Means are presented in ± SE. For hypothesis testing $P < 0.05$ was considered significant.

For the growth in nestlings, logistic growth curve was used (Starck and Ricklefs, 1998), by the given equation:

$$W = A/(1 + exp(- K(t – ti)))$$

Where W = the growth variable, A = asymptote, K = the growth rate constant, t = age of nestling, and ti = the inflection point of the growth curve. The logistic growth equations were fitted to the data using the nonlinear regression procedure of the SAS package version 9.1.

RESULTS

The body mass

Of the 48 nestling barn owls measured, only 32 were successfully measured up to day 49 while the rest died. Of the 32 nestlings, 12 were from the untreated control plot, eight from the warfarin treated plot, two from the brodifacoum treated plot and ten from the biorodenticide treated plot. Since only two nestlings in the brodifacoum treated plot survived during the measurements, the growth comparisons were made based on the age of these nestling with other nestlings in the other treatment plots.

From 226 measurements for nestlings from hatching up to fledging in the untreated control control plot (n = 12), 153 measurements in the warfarin treated plot (n = 8), 67 measurements in the brodifacoum treated plot (n = 2) and 174 measurements in the biorodenticide treated plot (n = 10), nestlings grew from 18.00 ± 0.37 g (n =6), 18.20 ± 0.58 (n = 5), 18.25 ± 0.49 (n = 4), 18.20 ± 0.37 (n = 5) at hatching, to a peak mass of 631.60 ± 12.96 g (day 46, n = 5), 597.30 ± 5.24 g (day 45, n = 3), 565.00 ± 16.05 g (day 43, n = 2) and 604.67 ± 16.00 g (day 45, n = 4) in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-1). Nestlings in control plot have a heavier body mass compared to the average adult body mass by 4.16% (545.90 ± 9.04, n = 10), and lighter by 3.71%, 7.86%, 2.55% for nestlings from warfarin, brodifacoum and biorodenticide treated plots respectively compared to adult body mass. Nestlings in control plot were heavier in body mass by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. Kruskal-Wallis test showed that there was no significant difference for body mass of nestlings in all treatments irrespective of days from day 1 to day 49 for the nestlings.

Table-1. Body mass (mean ± SE) of nestling barn owls in rodenticide treated areas.

Age (days)	**Body mass** (g)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	18.00 ± 0.37 ns	18.20 ± 0.58	8.25 ± 0.48	18.20 ± 0.37
7	73.67 ± 4.58 ns	84.00 ± 2.94	9.00 ± 3.52	81.00 ± 1.15
14	223.67 ± 11.99 ns	216.00 ± 13.50	218.33 ± 7.62	215.00 ± 11.53
22	379.80 ± 10.26 ns	359.33 ± 16.72	355.50 ± 21.56	364.75 ± 15.46
28	449.40 ± 10.16 ns	436.00 ± 9.30	428.50 ± 20.56	434.75 ± 15.17
34	546.80 ± 13.46 ns	536.67 ± 8.38	525.50 ± 24.57	546.00 ± 14.71
43	615.00 ± 7.75 ns	588.00 ± 16.04	565.00 ± 16.03	590.50 ± 10.60
49	568.60 ± 15.58 ns	525.67 ± 18.24	503.00 ± 13.03	532.00 ± 15.35

Generally, absolute rates of growth varied throughout the nestling period (Figure-1) and the most rapid rates occurred between days 10 - 35. Although the growth constant did not vary and ranging from 0.146 to 0.150 g per day, but the asymptote calculated using SAS Version 9.1 indicates the highest asymptote was found in nestlings from the control plot, followed by the biorodenticide, warfarin, and brodifacoum treated plots, respectively. The highest increase in body mass differ from one treatment to another, whereby the control plot was recorded on day 19.20 ± 0.26, in warfarin day 18.87 ± 0.31, in brodifacoum on day 17.99 ± 0.40, in biorodenticide on day 18.75 ± 0 (Table-2/ Figure-1).

Table-2. Body mass (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas.

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	613.5 ± 5.98	0.150 ± 0.004	19.20 ± 0.26
B	585.8 ± 6.62	0.146 ± 0.005	18.87 ± 0.31
C	554.9 ± 8.72	0.145 ± 0.006	17.99 ± 0.40
D	586.1 ± 6.60	0.147 ± 0.005	18.75 ± 0.30

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

The culmen length

The culmen length of nestling barn owls grew from 7.50 ± 0.10 mm (n = 6), 7.60 ± 0.10 mm (n = 5), 7.50 ± 0.13 mm (n = 4), 7.60 ± 0.10 mm (n = 5) in control warfarin, brodifacoum, and biorodenticide treated plots respectively at hatching to 22.70 ± 0.20 mm (n = 65, 22.00 ± 0.17 mm (n = 3), 21.50 ± 1.00 mm (n = 2) and 22.38 ± 0.37 mm (n = 4) for corresponding treatment plots at day 49. The culmen grew full length to reach the adult size (22.80 ± 0.17, n = 10) during the growth period when measurement were taken at day 49. Culmen length in the control plot was longer by 3.18%, 5.58%, and 1.43% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. Kruskal-Wallis test showed there was no significant difference for culmen length of nestlings in all treatments irrrespective of days, from day 1 to day 49 for the nestlings.

Patterns in growth of culmen were similar to that of body mass that exhibits a sigmoidal curve, where the culment grew slowly in the first 10 days and then rapidly to day 35, slowing down again to day 49. The asymptote of culmen length tend to be similar for nestlings in all treatments ranging from 22.926 ± 0.23 mm, 22.160 ± 0.20 mm, 22.040 ± 0.37 mm and 22.878 ± 0.23 mm for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from 0.110 ± 0.004 mm, 0.105 ± 0.004 mm, 0.102 ± 0.005 mm and 0.112 ± 0.005 mm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in culmen length was also quite similar from one treatment to another, ranging from day 10.768 ± 0.31, day 10.171 ± 0.28, day 10.125 ± 0.46, and day 10.500 ± 0.39 in nestlings from control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-4/ Figure-2).

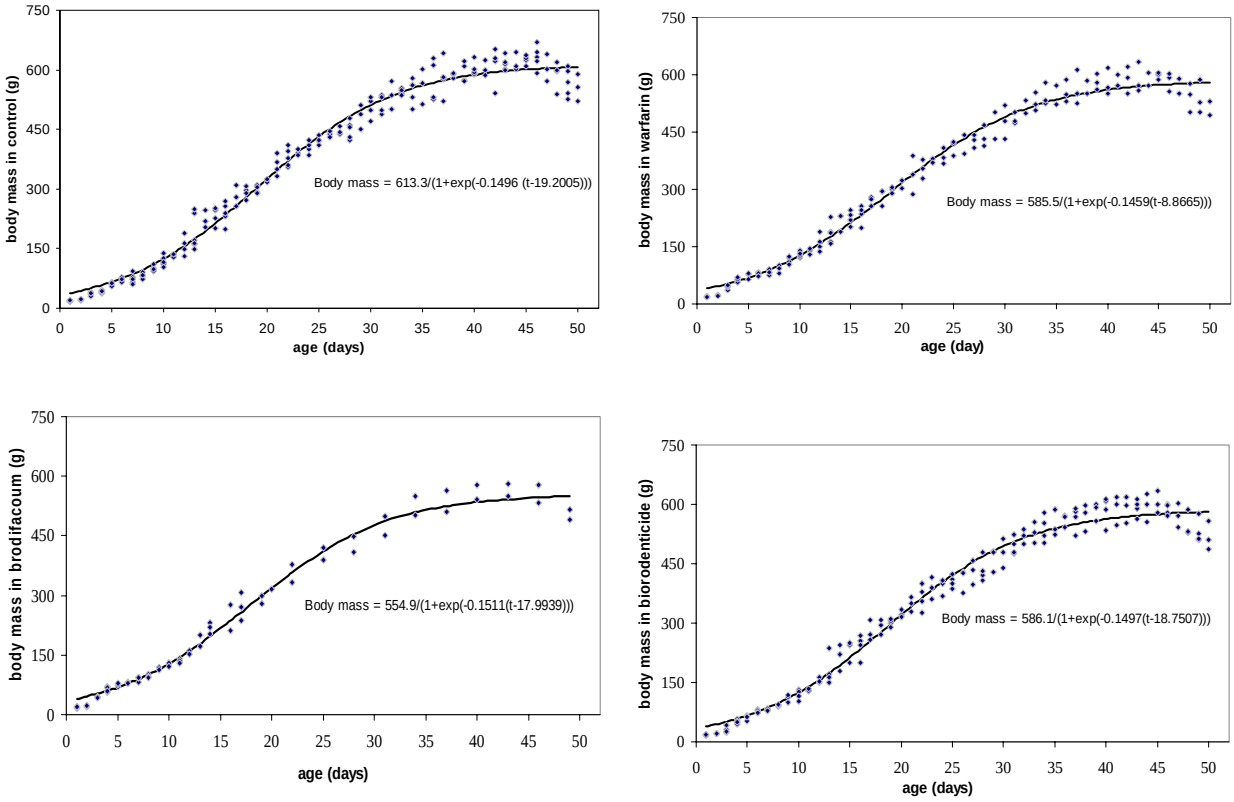

Figure-1. Logistic growth curve of the changes in body weight of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

Table 3. Culmen length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Culmen length** (mm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	7.50 ± 0.10 ns	7.60 ± 0.10	7.50 ± 0.13	7.60 ± 0.10
7	8.92 ± 0.15 ns	9.00 ± 0.16	9.00 ± 0.15	9.00 ± 0.18
14	13.00 ± 0.29 ns	12.83 ± 0.17	12.67 ± 0.17	13.17 ± 0.17
22	18.30 ± 0.20 ns	18.00 ± 0.29	18.00 ± 0.50	18.38 ± 0.23
28	20.60 ± 0.30 ns	19.33 ± 0.33	19.50 ± 0.50	20.75 ± 0.14
34	21.00 ± 0.45 ns	20.33 ± 0.17	20.25 ± 0.25	21.25 ± 0.25
43	21.90 ± 0.10 ns	21.33 ± 0.17	20.75 ± 0.75	22.00 ± 0.20
49	22.70 ± 0.20 ns	22.00 ± 0.17	21.50 ± 1.00	22.38 ± 0.37

Table-4. Culmen length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	22.926 ± 0.23	0.110 ± 0.004	10.768 ± 0.31
B	22.160 ± 0.20	0.105 ± 0.004	10.171 ± 0.28
C	22.040 ± 0.37	0.102 ± 0.005	10.125 ± 0.46
D	22.878 ± 0.23	0.112 ± 0.005	10.500 ± 0.39

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

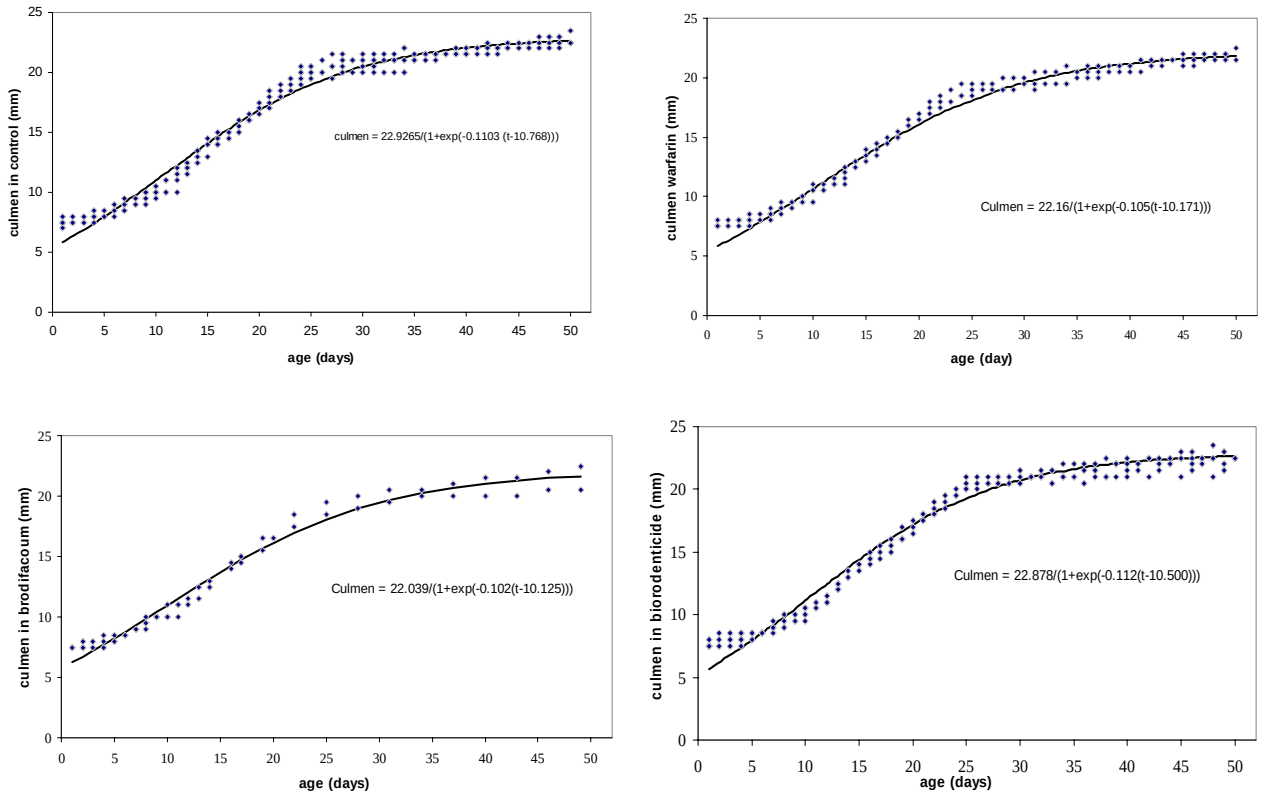

Figure-2. Logistic growth curve of the changes in culmen length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

The tarsus length

The tarsus length of nestlings grew from 16.33 ± 0.33 mm (n = 6), 16.70 ± 0.30 mm (n = 5), 16.00 ± 0.41 mm (n = 4), 16.20 ± 0.37 mm (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to 86.40 ± 0.81 mm (n = 5), 84.33 ± 1.20 mm (n = 3), 83.75 ± 1.00 mm (n = 2) and 83.50 ± 1.32 mm (n = 4) for the corresponding treatment plots in day 49 (Table-5). Same like culmen, tarsus length of chicks grew and reached adult size (86.45 ± 0.20, n= 10) during the growth period. Tarsus length in the control plot was longer by 2.45%, 3.16% and 3.47% compared to warfarin, brodifacoum and biorodenticide treated plots, respectively. The tarsus length in control plot was longer than rodenticides treated plot. However, there was no significant different irrespective of days when tested with Kruskal-Wallis analysis of variance.

Table-5. Tarsus length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Tarsus length** (mm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	16.33 ± 0.33 ns	16.70 ± 0.30	16.00 ± 0.41	16.20 ± 0.37
7	23.00 ± 0.37 ns	22.60 ± 0.24	22.33 ± 0.67	22.50 ± 0.31
14	46.00 ± 2.08 ns	46.33 ± 0.88	45.33 ± 1.45	44.33 ± 1.45
22	67.00 ± 1.58 ns	67.67 ± 0.33	69.00 ± 1.00	69.00 ± 0.91
28	78.40 ± 1.21 ns	78.00 ± 0.58	78.00 ± 3.01	77.50 ± 1.19
34	83.00 ± 1.22 ns	81.67 ± 1.20	80.00 ± 2.51	80.25 ± 0.85
43	85.40 ± 1.21 ns	83.00 ± 1.00	83.00 ± 2.00	82.50 ± 1.04
49	86.40 ± 0.81 ns	84.33 ± 1.20	83.75 ± 2.76	83.50 ± 1.32

The tarsus length grew slowly in the first seven days and then rapidly to day 30, slowing down again to day 50. The tarsus length reached asymptote around 30 to 35 days after nestling hatched, ranging from 86.93 ± 0.43

mm, 84.44 ± 0.45 mm, 84.39 ± 1.03 mm and 83.66 ± 0.52 mm for control warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from 0.14 ± 0.0043 mm, 0.15 ± 0.003 mm, 0.15 ± 0.006 mm and 0.15 ± 0.004 mm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in tarsus length differs from day 13.34 ± 0.15, day 12.86 ± 0.16, day 13.03 ± 0.30, and day 12.90 ± 0.18 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-6/ Figure-3).

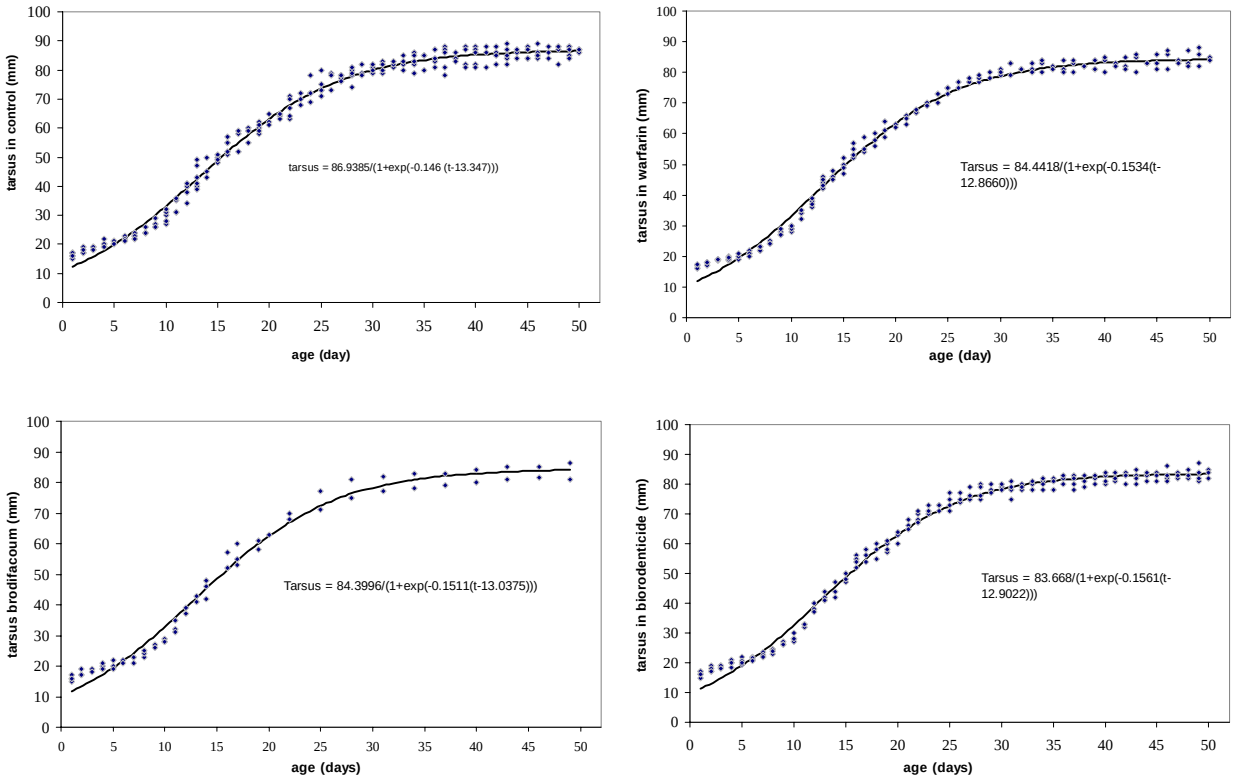

Figure-3. Logistic growth curve of the changes in tarsus length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Table-6. Tarsus length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	86.93 ± 0.43	0.14 ± 0.003	13.34 ± 0.15
B	84.44 ± 0.45	0.15 ± 0.003	12.86 ± 0.16
C	84.39 ± 1.03	0.15 ± 0.006	13.03 ± 0.30
D	83.66 ± 0.52	0.15 ± 0.004	12.90 ± 0.18

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

The wing length

The wing length of nestlings grew from 1.48 ± 0.03 cm (n = 6), 1.50 ± 0.04 cm (n = 5), 1.50 ± 0.04 cm (n = 4), 1.44 ± 0.05 cm (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to 26.02 ± 0.21 cm (n = 5), 25.93 ± 0.20 cm (n = 3), 22.05 ± 0.36 cm (n = 2) and 26.07 ± 0.23 cm (n = 4) for the corresponding treatment plots at day 49 (Table-7). Unlike tarsus and culmen that reached the adult size during the growth period, wing length still grew up to day 49, several days before fledging, and wing length was shorter by 15.77%, 16.06%, 28.62%, and 15.60% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size (30.89 ± 0.14, n=10). Wing length in brodifacoum treated plot was shorter by 15.26% compared to control plot.

Table-7. Wing length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	Wing Length (cm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	1.48 ± 0.03 ns	1.50 ± 0.05	1.50 ± 0.04	1.44 ± 0.05
7	3.30 ± 0.04 ns	3.24 ± 0.05	3.27 ± 0.03	3.20 ± 0.02
14	6.80 ± 0.12 ns	6.90 ± 0.03	6.90 ± 0.21	6.90 ± 0.17
22	14.96 ± 0.14 ns	14.87 ± 0.17	14.60 ± 1.20	14.80 ± 0.26
28	20.94 ± 0.26 ns	21.07 ± 0.18	19.30 ± 2.91	20.83 ± 0.17
34	24.08 ± 0.19 ns	23.77 ± 0.09	20.75 ± 3.97	23.97 ± 0.17
43	25.56 ± 0.19 ns	25.07 ± 0.17	21.55 ± 4.16	25.53 ± 0.15
49	26.02 ± 0.21 ns	25.93 ± 0.20	22.05 ± 4.36	26.07 ± 0.23

The asymptote reached by the wing length were 26.28 ± 0.11 cm, 25.86 ± 0.13 cm, 22.15 ± 0.23 cm and 26.24 ± 0.10 cm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. For the growth constant, ranging from 0.167 ± 0.003 cm, 0.169 ± 0.003 cm, 0.174 ± 0.005 cm and 0.165 ± 0.002 cm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in wing length was differ from one treatment to another, ranging from day 20.18 ± 0.11, day 19.99 ± 0.13, day 18.41 ± 0.24, and day 20.12 ± 0.10 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively.

Teratogenic signs showed by a nestling in brodifacoum treated plot, where up to 49 days old it had malformed primary feathers rendering it flightless (Figures 4 and 5). No nestlings in control, warfarin and biorodenticide treated plots shown teratogenic sign as showed by nestling in brodifacoum treated plot.

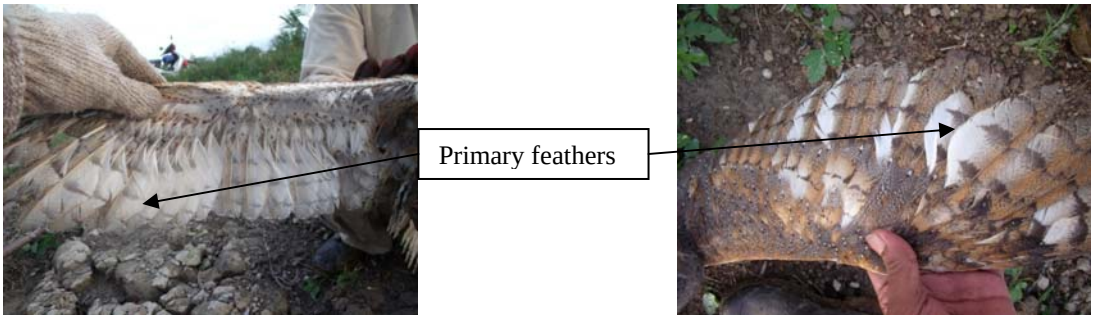

Figure-4. Normal nestling.

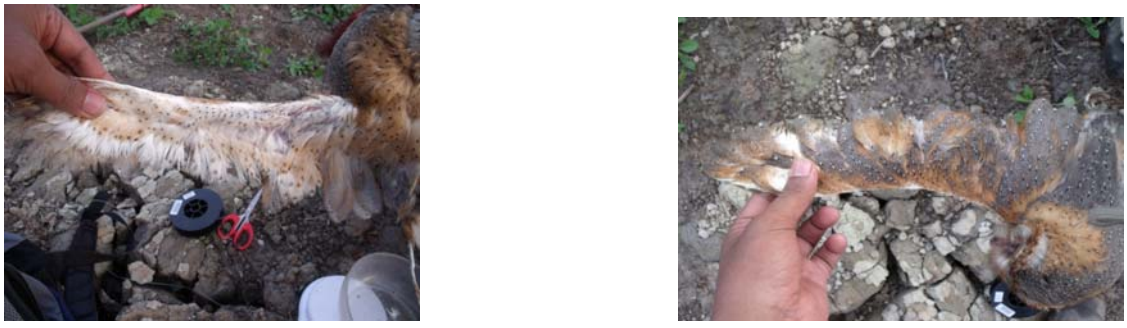

Figure-5. Teratogenic sign showed by nestling in brodifacoum treated plot that has no primary feathers.

The tail length

The tail length of nestlings grew from 0.00 ± 0.00 cm in all treatment at hatching to 11.62 ± 0.17 cm (n = 5), 11.47 ± 0.15 cm (n = 3), 9.50 ± 1.00 cm (n = 2) and 11.85 ± 0.21 cm (n = 4) for control, warfarin, brodifacoum and biorodenticide treated plots at day 49 (Table-9). Same like wing length, the tail length still grew up to day 49. Tail length was shorter 13.73%, 14.85%, 29.47%, 13.88% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size (13.47 ± 0.14, n = 10). The tail length of nestlings in brodifacoum treated plot also shorter by 18.24% compared to nestling in control plot.

Table-8. Wing length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	26.28 ± 0.11	0.167 ± 0.003	20.18 ± 0.11
B	25.86 ± 0.13	0.165 ± 0.003	19.99 ± 0.13
C	22.15 ± 0.23	0.174 ± 0.005	18.41 ± 0.24
D	26.24 ± 0.10	0.165 ± 0.002	20.12 ± 0.10

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

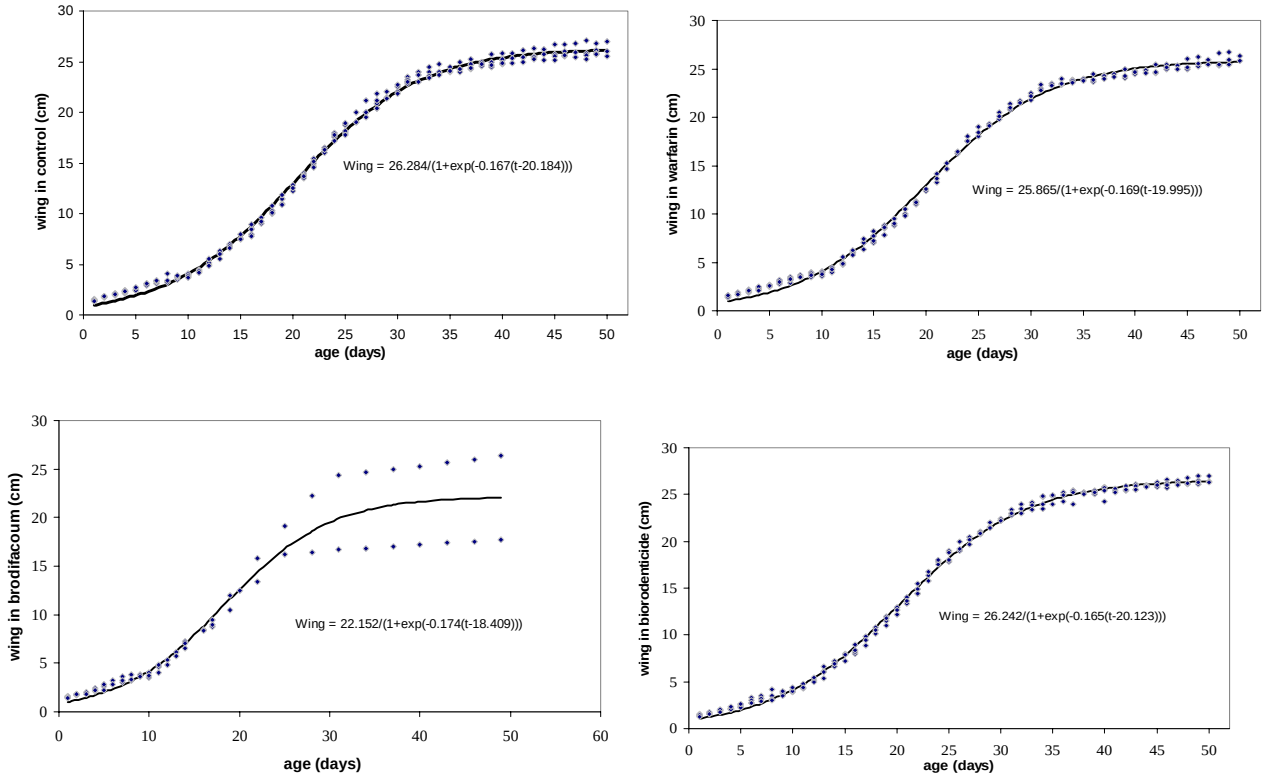

Figure-6. Logistic growth curve of the changes in wing length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Table-9. Tail length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Tail length** (cm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	0.00 ± 0.00 ns	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	0.35 ± 0.02 ns	0.46 ± 0.05	0.37 ± 0.05	0.43 ± 0.02
14	1.23 ± 0.09 ns	1.23 ± 0.03	1.27 ± 0.03	1.30 ± 0.06
22	4.78 ± 0.07 ns	4.83 ± 0.08	4.80 ± 0.10	4.80 ± 0.16
28	7.44 ± 0.10 ns	7.03 ± 0.14	7.50 ± 0.10	7.25 ± 0.09
34	9.68 ± 0.12 ns	9.37 ± 0.18	8.85 ± 0.65	9.43 ± 0.20
43	10.94 ± 0.12 ns	10.87 ± 0.15	9.20 ± 0.80	10.87 ± 0.13
49	11.62 ± 0.17 ns	11.47 ± 0.15	9.50 ± 1.00	11.60 ± 0.17

The nestling's tail length reached asymptote between 40 - 45 days with the constant growth rate ranging from 0.19 ± 0.004 g per day, 0.17 ± 0.004 g per day, 0.24 ± 0.005 g per day and 0.17 ± 0.004 g per day for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in tail length differs from one treatment to another, ranging from day 24.15 ± 0.16, day 25.03 ± 0.19, day 21.82 ± 0.12, and day 24.61 ± 0.16 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-10/ Figure-5).

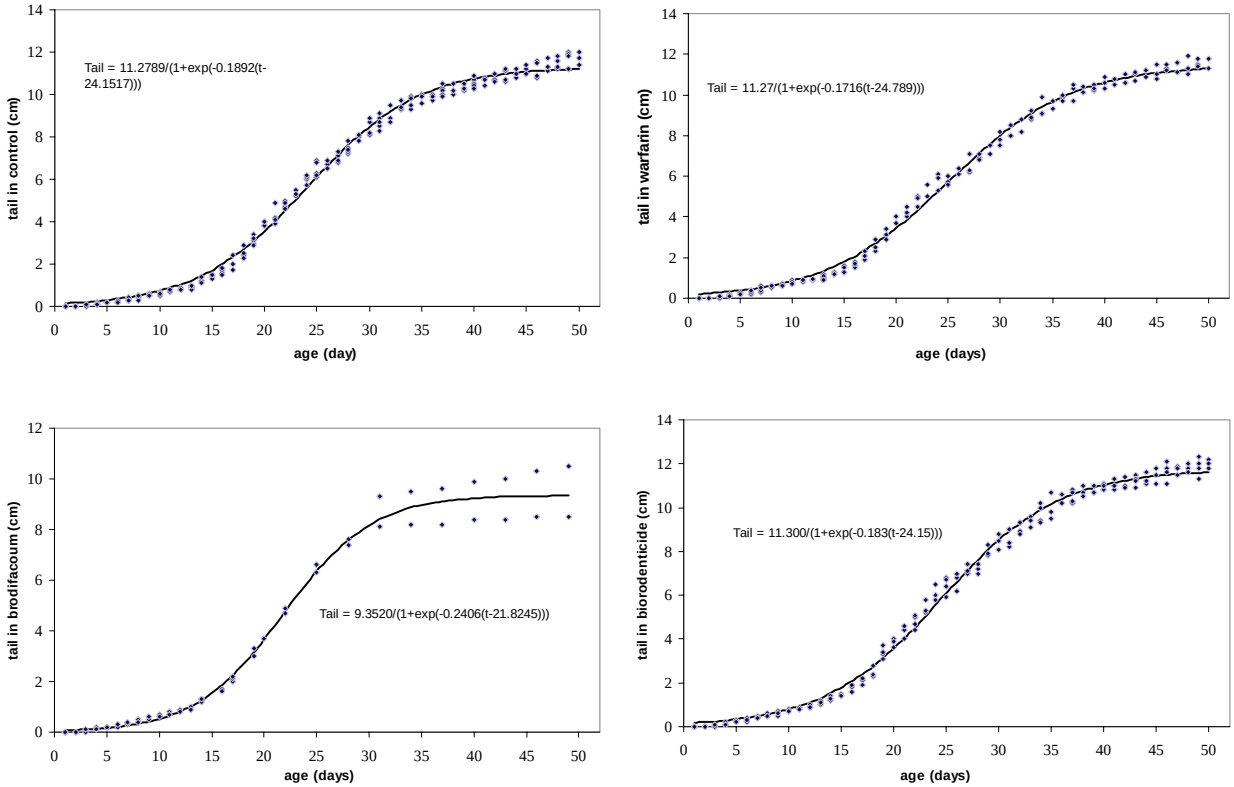

Figure-7. Logistic growth curve of the changes in tail length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

Table-10. Tail length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	11.28 ± 0.08	0.19 ± 0.004	24.15 ± 0.16
B	11.46 ± 0.10	0.17 ± 0.004	25.03 ± 0.19
C	9.35 ± 0.09	0.17 ± 0.004	24.61 ± 0.16
D	11.74 ± 0.05	0.24 ± 0.005	21.82 ± 0.12

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

DISCUSSIONS

Growth in bird nestlings has been frequently described using weight versus age curves. Growth in birds generally follows a sigmoidal curve, where a small initial increase is followed by a period of relatively rapid growth before leveling off. Information of most interest is the form of the growth curve, its final magnitude or asymptote, and the rate at which it traversed (O'Connor, 1984). It has long been known that proportional differences must exist among growing anatomical parts as a result of genetic, physiological or environmental variation (Starck and Ricklefs, 1998). The constant growth rate in body mass of nestling of barn owls, *T. alba javanica* in untreated control plot was similar as previously reported by Lenton (1984) i.e., K = 0.150-0.162 , and with African subspecies *T. alba affinis* (K = 0.151) (Wilson *et al*, 1987), but higher than Indian barn owl, *T. alba stertens* (K = 0.132) (Nagarajan *et al*., 2002). In rodenticides treated plot, body mass of nestlings is lower than in control plot. This was probably due the stable

number of rat prey in control plot where the adult barn owl can deliver enough food to females and nestlings regularly, in contrast to rodenticide treated plots where rat population experience a crash due to baiting campaign. A study done by Wood (1984) and Liau (1990) found that in unbaited area, the rat population varied between 200 and 600 per ha, with slow fluctuations. However, rat population drop to less than 150 per ha after baiting, and only established six month after control (Wood and Fee, 2003).

The body mass of nestlings in rodenticide treated plots especially brodifacoum reached a lower asymptote than control plot. Although some nestlings survived to fledging age, others were found dead in the nest box. This is because in rodenticide treated plots the rat populations were not as abundant as in the control plot and encourage the males to travel farther and take a longer time to bring rat prey to the nestling. When food is limited the older nestling will out compete the younger siblings for food depriving the latter of food bringing down the average body mass. A study by Durant and Handrich (1998) showed that nestlings have the same body mass when food given is reduced by 17% when compared to nestlings fed enough and show the same pattern for linear growth and fledging. However, when food is reduced to more than 30% than usual, the nestlings showed lower fat accumulation when compared to normal fed nestlings (Lacombe *et al*., 1994).

The culmen and tarsus length in control plot grew rapidly in the first 3 weeks and reached the adult size in the growth period, quite similar to that reported by Wilson *et al* (1987) and Nagarajan *et al.* (2002) where they reported higher growth rates for these bodily parts for young nestlings in Central Mali and India and can reach adult size before fledging. The rapid growth of culmen was also reported in the spotted owl (*Athene brama brama*) (Kumar, 1983). Faster growth of these body parts may be a direct reflection of the use of these organs during the nesting period and immediately after fledging (Holcomb and Twiest, 1968). Rapid leg growth rates as evidenced by tarsus and talon growth rate are another feature of growth in nestling barn owls. Rapid growth of the legs considered by Nagarajan *et al.* (2002) as a selective advantage in competition within broods and also important in post fledging foraging activities, such as collection and handling of food items. In contrast to culmen and tarsus, wing and tail length was shorter than adult size until fledging. Wilson *et al.* (1987) and Nagarajan *et al.* (2002) reported that tail and wing will continue to grow after fledging. The tail and wing would still increase in length after the bird had left the nest and the full ability of young barn owls to catch their own prey would not be achieved until some time had elapsed after leaving the nest (Wilson *et al.*, 1987).

Besides receiving less food than the nestlings in the control plot, nestlings in rodenticide treated plots also face the risk of secondary poisoning by rodenticide residues. If the parents bring home rats that had consumed baits, the young would be exposed to the ingested rodenticide especially brodifacoum, risking them to secondary poisoning. Brodifacoum acts by inhibiting the normal synthesis of vitamin K in the liver (Hadler and Shadbolt, 1975), resulting in an increase in blood clotting time to the point where haemorrhaging occurs (Eason *et al*., 2002). A study showed that the potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to non-target animal either by direct consumption or from build-up of rodenticide residues from indirect consumption of baits (Shore *et al*., 1999; Dowding *et al*., 2010). Mendenhall and Pank (1980) reported that five of six *T. Alba* fed with rats poisoned with brodifacoum died. If the larger adults succumb from rodenticide poisoning the risk to the nestlings would be definitely greater.

Sarcocystis singaporensis is highly host-specific and only lives in the boid snake (*Python reticulatus*) and rodents of the genera *Rattus* and *Bandicota*. The infection of rats is by the sporozoites which eventually invades the muscles to from characteristics cyst in the striated muscles. After inoculation of a lethal quantity of sporocysts, the number of merozoites, the infective stage of the pathogen increase enormously around day 11 post infection especially in the lungs. This induces a fatal pneumonia (Jakel *et al*, 1996).

Warfarin, the first generation anticoagulant, is less toxic than brodifacoum. It is not persistent, and readily metabolized and excreted, and is not retained in the liver beyond 2-4 weeks, while brodifacoum is retained in the liver for 6-12 months (Eason *et al*., 2002). Several studies have shown that birds were almost completely resistant to the effects of warfarin (Papworth 1958). The same indication was also shown by the tawny owl (*Strix aluco*) when given mice that have consumed warfarin on alternate days for three months with no death or apparent behavioral changes (Townsend *et al.*, 1981). Lenton (1984) estimated barn owl nestlings need to consume at least ten medium sizes rats (80g) before a lethal level is reached.

In these study nestlings in rodenticide treated plots, especially brodifacoum showed shorter and lighter measurements in all five anatomical features: body mass, culmen, tarsus, wing and tail length. Previous workers showed that some birds have shorter anatomical parts and lighter in body mass if they are exposed to pesticides or if lived in polluted area. The screech owls (*Otus asio)* administered with fluoride at 40 ppm resulted in a significantly smaller egg and shorter tarsus length (Hoffman *et al*., 1985). The nestlings of the great (*Parus major*) that lives at large non-ferrous smelter and exposed to large amounts of heavy metals have a body mass significantly reduced at the most polluted site although tarsus length, wing length and haematocrit values did not differ significantly among study sites (Janssens *et al*., 2003). The nestling zebra finch (*Taeniopygia guttata*) that were orally dosed with monosodium methanearsonate (MSMA) for 20 days from hatching to fledging showed high mortality if given 24 mg/g, while surviving nestlings showed accumulation of arsenic in blood and specific

tissues, and decreased tarsus length and wing length upon fledging (Albert *et al.*, 2008).

Teratogenic effect was also evidenced in one of the nestling in brodifacoum treated area where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Several pesticides studies also found teratogenic effect on growth and development of birds. The chicken embryos were exposed *benzo [a] pyrene (BP)* via the yolk sac route resulted in retarded growth, as reflected by lower embryonic body weight besides reduced bill length. Abnormal survivors also showed remarkably twisted legs with shortening of the bones, abdominal oedema, haematomas, blisters and a short neck (Anwer and Mehrotra, 1988). Fry (1995) also reported organochlorine, organophosphate, petroleum hydrocarbons, heavy metals, and polychlorinated biphenyls (PCBs) disrupt physiological effects at several levels on birds, including direct effects on breeding adults as well as developmental effects on embryos. The effects on embryos include mortality or reduced hatchability, failure of chicks to thrive (wasting syndrome), and teratological effects producing skeletal abnormalities and impaired differentiation of the reproductive and nervous systems through mechanisms of hormonal mimicking of estrogens. The eggs of Mallard (*Anas platyrhynchos*) that were treated by *Phenyl phosphonothioic acid-o-ethyl-O-[4-nitrophenyl] ester (EPN)* resulted in impaired embryonic growth and was highly teratogenic: 37-42% of the surviving embryos were abnormal with cervical and axial scoliosis as well as severe edema. Brain weights were significantly lower in EPN-treated groups at different stages of development including hatchlings. Hatchlings from *EPN* treated eggs were weaker and slower to right themselves compared to untreated hatchlings (Hoffman and Sileo, 1984).

CONCLUSIONS

Nestlings in rodenticide free area showed consistently heavier body mass and longer in culmen, tarsus, wing and tail length compared to rodenticide treated plots. This was associated with nestlings in rodenticides free getting sufficient food during the growth stage. The food shortage in rodenticide treated plots affect the growth of nestling and exposed to a greater risk of death especially for nestlings less than 20 days old if food shortage continues. Nestlings in brodifacoum treated area did not only face the risk of food shortages but also the risk of secondary poisoning as a result of consuming bait ingested rats. Even one nestling has teratogenic signs where it has no primary feathers in its wings rendering it flightless and the size of the tail is shorter than nestling in rodenticide free area. However, nestlings in warfarin and biorodenticide treated plots have comparable anatomical parts except body mass if compared to rodenticide free area, an indication that there was no apparent evidence of secondary poisoning effect of warfarin and biorodenticide on nestling of barn owls.

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Comparison of the breeding performance of the barn owl Tyto alba javanica under chemical and bio-based rodenticide baiting in immature oil palms in Malaysia

Citation

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Abstract

The breeding performance of barn owl, Tyto alba javanica, in areas treated with rodenticides in immature oil palms in Malaysia was investigated. Four plots were established, each at least 100 ha in size and treated with warfarin, brodifacoum, a biorodenticide (Sarcocystis singaporensis) and a non-baited control plot. Three rat baiting campaigns, which coincided with the barn owl breeding season, were carried out in October 2008, February and March 2009, and in October 2009. The nest boxes were distributed at a mean density of one unit per 25 ± 3.83 ha. The clutch size, hatching and fledging rates of barn owls in each plot was monitored monthly from September 2008 to January 2010. There was no significant difference in mean clutch size for all four treatments. The lowest percentage of hatching success was recorded in the brodifacoum-treated plot in all three breeding seasons. Fledging success was highest in the control plot, followed by the S. singaporensis-, warfarin- and brodifacoum-treated plots. The mean clutch size and mean hatching success was not significantly correlated with mean rat damage (clutch size, $r = 0.754$, $p > 0.05$; mean hatching success, $r = 0.832$; $p > 0.05$). The mean fledging success was significantly correlated with mean rat damage ($r = 0.969$; $p < 0.05$). Brodifacoum achieved the lowest level of rat damage but not significantly lower than warfarin and S. singaporensis. This indicates that S. singaporensis is a better rodenticide than warfarin and brodifacoum in controlling rats and yet achieved the highest reproductive rates in the baited areas as reflected by the rate of fledging success.

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Every dollar spent in CA restaurants contributes
$1.89
to the state economy

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restaurant & foodservice jobs

$33.1B
total taxes paid by restaurants in the state

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restaurant & foodservice sales

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restaurants are
small businesses

Source: National Restaurant Association, based on data from the Bureau of Labor Statistics and U.S. Census Bureau; 2024 data

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CALIFORNIA RESTAURANT INDUSTRY IMPACT

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Alex Padilla (D) Adam Schiff (D)	**86,779**	**1,490,100**

	U.S. REPRESENTATIVES	EATING & DRINKING PLACES Estab.	Employees*		U.S. REPRESENTATIVES	EATING & DRINKING PLACES Estab.	Employees*
1	Doug LaMalfa (R)	1,445	23,079	27	George Whitesides (D)	1,187	21,523
2	Jared Huffman (D)	2,014	28,741	28	Judy Chu (D)	2,036	31,368
3	Kevin Kiley (R)	2,080	39,300	29	Luz M. Rivas (D)	1,102	13,921
4	Mike Thompson (D)	1,707	33,448	30	Laura Friedman (D)	2,567	49,878
5	Tom McClintock (R)	1,682	27,543	31	Gilbert Ray Cisneros, Jr. (D)	1,586	22,948
6	Ami Bera (D)	1,270	21,288	32	Brad Sherman (D)	1,935	33,405
7	Doris O. Matsui (D)	1,589	26,366	33	Pete Aguilar (D)	1,134	19,929
8	John Garamendi (D)	1,155	15,186	34	Jimmy Gomez (D)	2,000	28,690
9	Josh Harder (D)	1,195	19,556	35	Norma J. Torres (D)	1,572	26,623
10	Mark DeSaulnier (D)	1,468	23,111	36	Ted Lieu (D)	2,564	55,012
11	Nancy Pelosi (D)	3,712	57,791	37	Sydney Kamlager-Dove (D)	1,428	22,932
12	Lateefah Simon (D)	2,058	25,557	38	Linda T. Sánchez (D)	1,628	21,832
13	Adam Gray (R)	1,021	15,152	39	Mark Takano (D)	1,130	20,153
14	Eric Swalwell (D)	1,424	20,253	40	Young Kim (R)	1,579	26,566
15	Kevin Mullin (D)	1,832	27,761	41	Ken Calvert (R)	1,559	36,041
16	Sam T. Liccardo (D)	1,936	31,135	42	Robert Garcia (D)	1,703	32,416
17	Ro Khanna (D)	2,187	32,737	43	Maxine Waters (D)	1,264	22,036
18	Zoe Lofgren (D)	1,427	20,410	44	Nanette Diaz Barragán (D)	1,302	19,520
19	Jimmy Panetta (D)	1,880	32,956	45	Derek Tran (D)	2,159	33,437
20	Vince Fong (R)	1,400	25,798	46	J. Luis Correa (D)	1,829	37,318
21	Jim Costa (D)	1,133	16,831	47	Dave Min (D)	2,339	51,464
22	David G. Valadao (R)	1,000	16,114	48	Darrell Issa (R)	1,346	31,913
23	Jay Obernolte (R)	1,213	19,017	49	Mike Levin (D)	1,742	35,209
24	Salud O. Carbajal (D)	2,208	40,354	50	Scott H. Peters (D)	2,546	61,580
25	Raul Ruiz (D)	1,092	20,202	51	Sara Jacobs (D)	1,885	30,646
26	Julia Brownley (D)	1,385	26,490	52	Juan Vargas (D)	1,146	17,570
					TOTAL	**86,779**	**1,490,100**

*California's 1,490,100 eating and drinking place jobs represent the majority of the state's total restaurant and foodservice workforce of 1,853,800 jobs, with the remainder being non-restaurant foodservice positions.

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Source: National Restaurant Association, based on data from the Bureau of Labor Statistics and U.S. Census Bureau; 2024 data

RAPTORS ARE THE SOLUTION

Menu

Poison Free 2023 (Queen Anne Pilot)

PARKER ECO PEST CONTROL

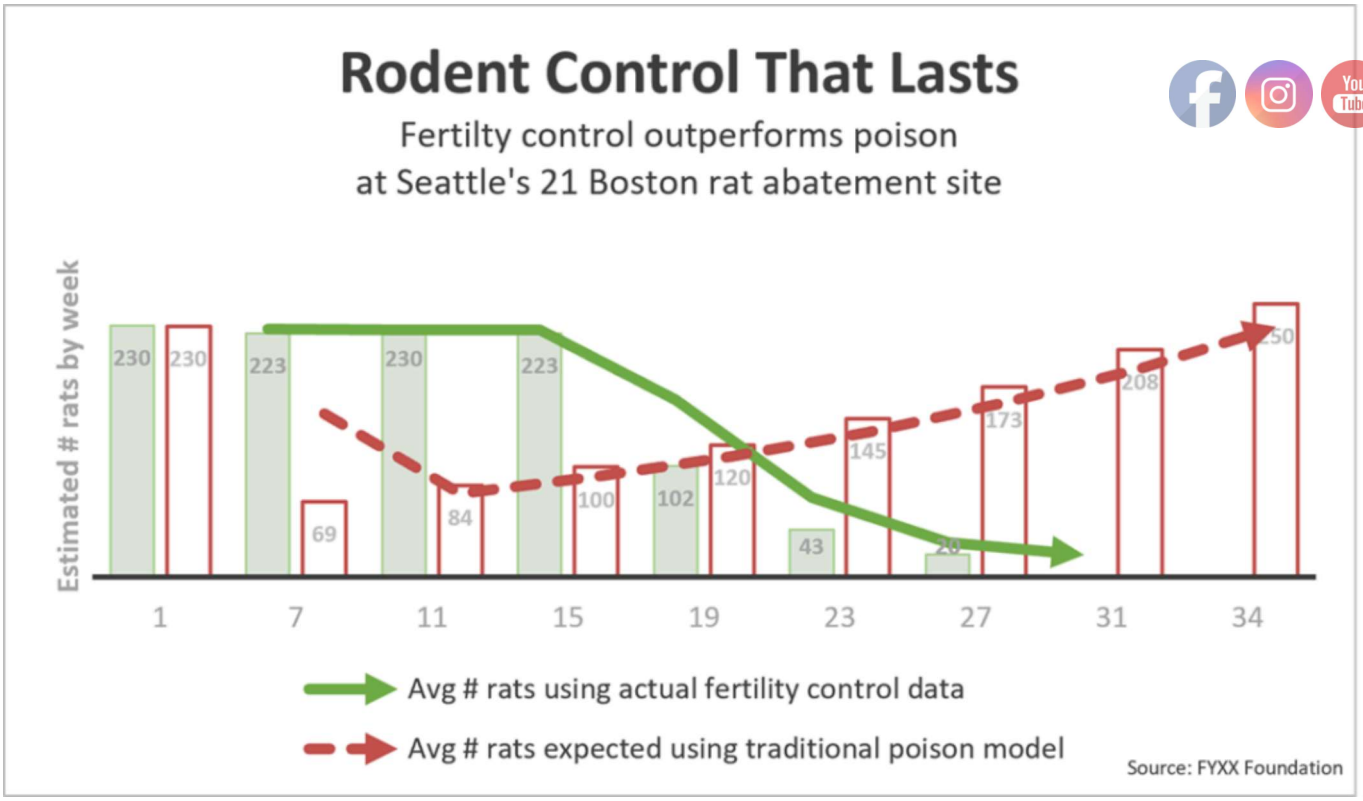
RAPTORS ARE THE SOLUTION SEA-RATS

POISON FREE BY 2023 OWL WISE LEADER

An Upper Queen Anne, Seattle, mixed-use business district demonstration pilot study managed by Raptors Are The Solution to illustrate cost savings and effective rat population management using a non-toxic, rat birth control solution called ContraPest®, as a replacement for a certain class of rat poisons that are harming and killing non-target wildlife and pets. We were able to reduce the rat population by 91 percent on this site in just a few months.

Read the latest news coverage about this project.

Queen Anne is working to become a showcase neighborhood for other communities to follow in their own poison-free rat birth control campaigns. The pilot study team aims to have its documented, successful protocols available for others to use, in the Community and Pest Control Playbook later this year.



A note about our inaugural Queen Anne demonstration pilot study participant TWENTY-ONE BOSTON

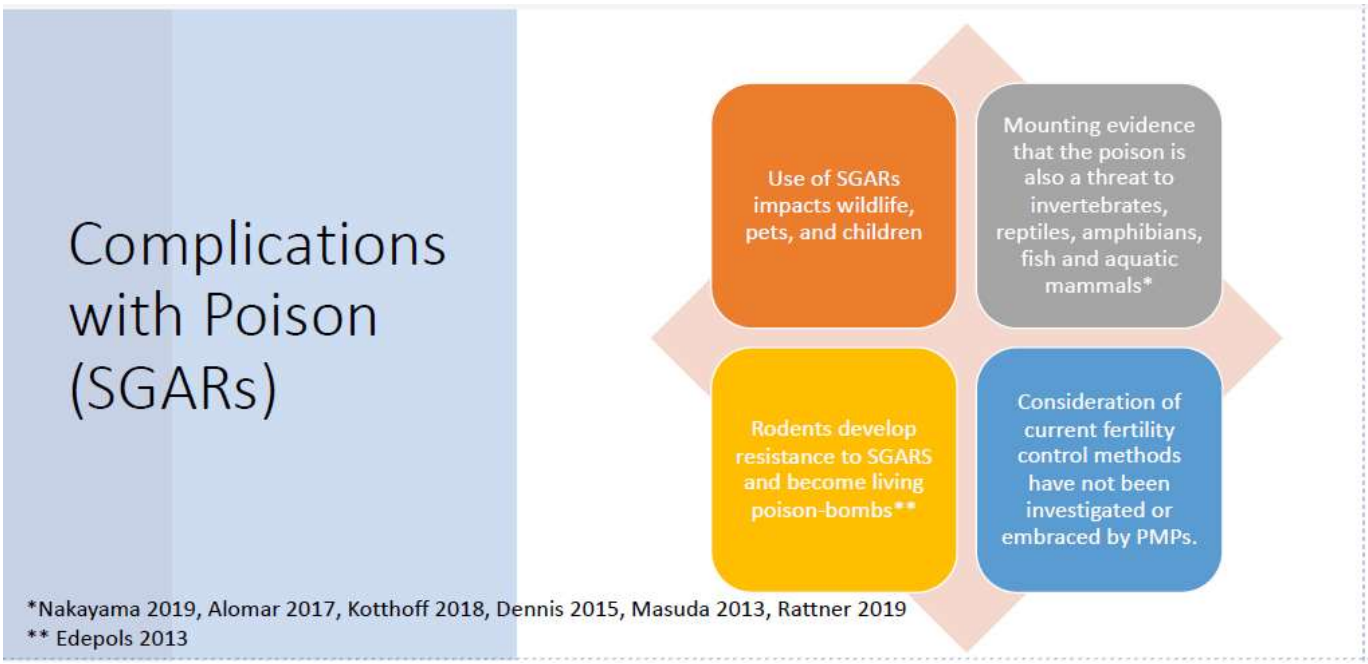
Demolition of Queen Anne's Safeway grocery store happened to make room for 325 apartments situated on top of a new 50,000 square foot grocery store and underground parking**.** Per the Seattle department of construction and inspections, the TWENTY-ONE BOSTON project was required to set out rat poison bait stations prior to ground disturbance caused by demolition. Queen Anne Pilot Study's first participant was developer and landowner Maria Barrientos, marking Seattle's first developer to use a non-toxic fertility control rat abatement strategy for demolition. We are proud to be reaching this trailblazing milestone together as we create a safer urban habitat for wildlife and pets.

At the 7th month mark of managing the future build site's resident Norway Rat population with rat birth control, the pilot study achieved, and held steady, a 91% reduction of the original rat population. The construction superintendent went so far as to remark that he's never demolished a building where he didn't see a single rat. Typically, legacy food-centric buildings are infested with rats by the time demolition happens. The project team received no complaints of migrating rats from the demolition site and nearby businesses benefited greatly from the rat birth control deployed at the Safeway site, taking wintertime rat activity to zero.

See the chart above, which illustrates the pilot study's rat birth control success compared to traditional blood thinning rat poison performance, which has a rebound effect on rat popula after the 4th generation. This happens because rats learn that their colony mates die after e

the bait and stop taking it, thereby returning to original rat population numbers because exponential reproductive growth of rats can no longer be managed with bait that is avoided.

The Problem With Blood-thinning Rat Poisons



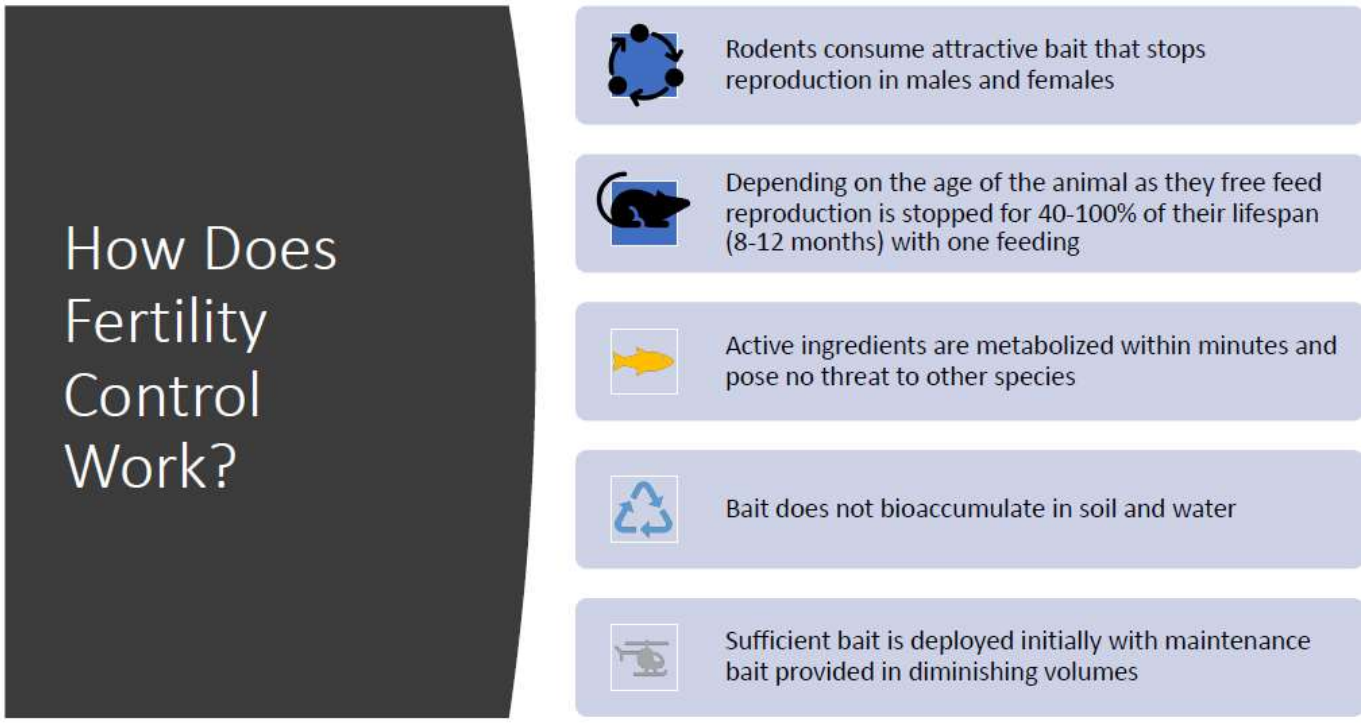
Source: FYXX Foundation

What can we do to stop anticoagulants from infiltrating the food web and killing non-target species?

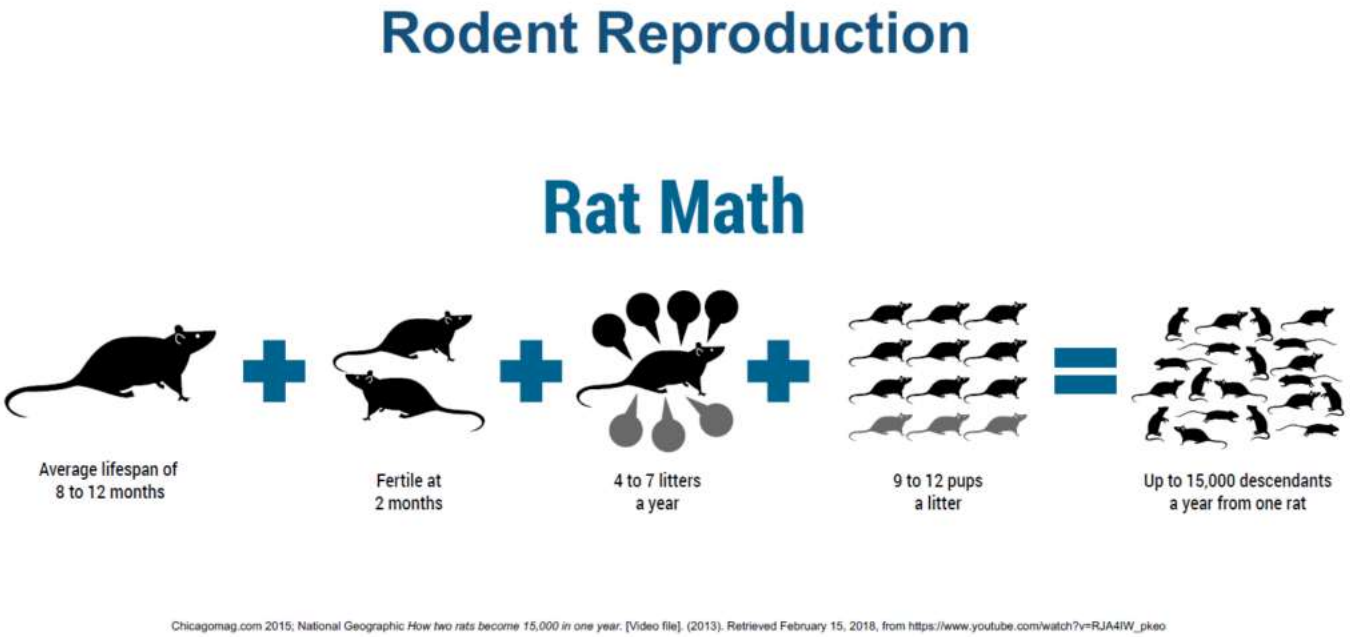
Replace commercially used blood-thinning rat poisons that are killing non-target wildlife and pets with an effective, non-toxic rat birth control rodent management solution, while improving sanitation and exclusion.

During a year-long pilot study, a mixed-use business district in Washington D.C. decreased its rodent population 99% by utilizing the rat birth control solution, called ContraPest®, which we are using for the Queen Anne pilot study.

Extensive scientific studies have shown that ContraPest® does not bioaccumulate inside the body of non-target species (it is not a hormone) and therefore does not impact predators that eat rats that have ingested this product.



Source: FYXX Foundation



Chicagomag.com 2015; National Geographic *How two rats become 15,000 in one year.* [Video file]. (2013). Retrieved February 15, 2018, from https://www.youtube.com/watch?v=RJA4IW_pkeo

How Deployment and Monitoring of a Business District Pilot Study Could Work

An eco-conscious pest management partner and activist team should:

- Ensure that ALL poison bait stations are properly disposed*;
- Deploy rat birth control bait stations throughout the alleyways and non-toxic rat deterrents inside businesses, if needed;

- Clean and monitor the bait stations on a monthly basis for consumption, and refill as needed. The amount of necessary fertility control product will decrease over time as the rat population decreases, thereby decreasing pest control costs for business owners.
- Collect bait consumption data for analysis that shows population decline.
- Address business concerns around maintenance of the bait stations or the very unlikely case of increased rat activity.
- Our current goal is to remove blood-thinning poisons from our Upper Queen Anne business district (encompasses Queen Anne Ave N., Boston to Galer) which covers 735,000 square feet of commercial space with rat birth control for an estimated monthly cost is $2,600 a month. If every business in the area participates it breaks down to just $18 per business per month.

Get Involved

Are you an Upper Queen Anne business that would like to participate in the pilot study, or someone who would like to remove rat poisons from their Seattle neighborhood? Call or e-mail Tanea Stephens at 206-579-4545 or searatschapter@gmail.com to get started.

Residential Rat Birth Control

People we talk with want to rid their homes of blood-thinning rat poisons. Because there is so much demand, beginning summer 2022, "In Harmony" will offer a nationwide residential DIY rat birth control subscription service. To be placed on a list for more information once it becomes available, sign up here. For those who want a hands-off approach to managing rats, Parker Eco Pest Control now offers Seattle metro residential full service rat birth control management. To get started with residential full service rat birth control, sign up here.

***Note**: when you decide to ditch rat poison for sanitation, exclusion, and non-toxic rodent control products, NEVER throw your old poison bait boxes in the garbage where they end up in the landfill, potentially poisoning rats, eagles, vultures, and other wildlife. If your pest control company does not pick up your old bait boxes, and you are in Seattle, send an e-mail to searatschapter@gmail.com to request a free bait box pick-up and drop-off to the King County Toxic Waste Disposal facility.

The Genesis Story

Tanea Stephens, a RATS Volunteer and Queen Anne resident became concerned about rodenticides in her neighborhood when a rare Snowy Owl visitor set up residence near the business district in October 2020. She took inventory of the toxic rat poisons and found 70 AR bait boxes along the ½ mile stretch of Upper Queen Anne's business district alleyways. After becoming the Washington state chapter representative for RATS and brainstorming with pilot study partner FYXX Foundation, the idea for Poison Free By 2023 was born. If the study garn the anticipated results, Queen Anne will become the showcase neighborhood for our ***Poison Free***

by 2023 citywide campaign and provide a case study that shows rats can be effectively managed without lethal poisons that harm non-target wildlife, pets and children.

Update: One year after the Queen Anne departure, Urban Raptor Conservancy reports that it tested 29 pellets that the Snowy owl cast onto rooftops and beneath roost trees and rodent bones were abundant in the pellets; a few were tested for toxicology and found evidence of anticoagulant rodenticides.

You can learn more about: our Snowy Owl visitor in this Queen Anne & Magnolia News Article; the pilot study in another Queen Anne & Magnolia News feature and Stranger Article; and a Queen Anne & Magnolia News feature on the developer deployment.

Top 3 False Claims by Pest Control Companies

FALSE: The poisons we use are safe. There is no proof that our poisons kill non-target wildlife.

FACT: There is no such thing as a safe rat poison. Current bait box poisons are infiltrating the entire food web and not only killing rats. There have been hundreds of scientific studies showing accumulation of rat poison in species of all kinds. Children under age six and domestic pets are known to have been poisoned.

FALSE: If we can't use anticoagulant rodenticides (ARs), there will be a public health crisis.

FACT: Improving sanitation and installing exclusion helps remove rat attractants, and there are non-toxic alternatives that can be used to manage rodent populations. We are only asking people and companies to stop using four of the most deadly AR poisons.

FALSE: Rats consume poison inside the box and therefore it cannot poison children or pets.

FACT: The loose, often bright blue bait is consistently found outside bait boxes. Rats carry rat poisons outside boxes where children and pets are attracted to them. Poisoned rats eat the bait both inside and outside the box. They leave the boxes, sickened and sluggish, becoming easy prey for raptors and other wildlife.

RAPTORS ARE THE SOLUTION

Menu

Got Rats?

Check out our "Got Rats" poster series for tips on controlling rats without poison.

Our **Got Rats?** posters, created with the help of experts including a pest control company, highlight numerous ways to keep rodents away without using poison and other harmful methods. Click links to share, download, or print!

Got Rats?

Got Restaurant Rats?

Got Rats in Your Crawl Space?

Got Rats in Your Barn?

Got Rats in Your Car?

Got Rodents on your Family Farm?

Raptors are the solution but... they can't be the entire solution. There are a variety of effective alternatives to poison. We recommend taking an "integrated pest management" approach to rodents that emphasizes exclusion and sanitation.

Rodent fertility control is a promising solution. Learn more about it.

Preferred pest control companies that do not use poison

For large scale (warehouse/industrial) rat issues

Preferred pest control products

Start by finding their source of food, shelter, and water and exclude them from it, or hire a company to help you. One of the primary attractants and sources of food for rodents is trash and waste. More tips.

Follow our best practices guide to keep rodents out of dumpsters and waste in!

Tips for restaurants

Tips if you have rats in your crawl space

Other tips on discouraging rats:

- Remove invasive ivy—it's a luxury hotel for rats. They thrive in it! Replace with native plants that offer habitat for other wildlife.
- Pick up bird seed waste in your yard. Instead of bulk seed, use *seed blocks or cakes* that leave less seed on the ground.
- Do not leave pet food out.
- Make sure garbage bags are tied tightly and secured.
- Backyard chicken coops attract rats. Consider installing rat-proof flooring in your coop.
- Consider installing a barn owl box—but ONLY if everyone in your neighborhood commits to not using poison. See the Hungry Owl Project for more information and tips.
- Orkin now offers a shield for your home that protects against rat entry.

For information about gopher problems, check out Gophers Limited.

A few warnings:

Any time you see a "bait box" with an exit hole—beware. If the box contains poison, it should be labeled as such. If it contains a trap instead, it must be labeled—otherwise, the box likely includes

poison.

Rats and mice can “check in,” eat poison, and then check out, easily becoming food for a hawk, an owl, other wildlife, or your pet cat or dog. Bait boxes are **not** safe for wildlife unless you get them from a company that uses snap traps inside them. (Most do not.) They should **never** be used out in the open where songbirds can get caught in them.

Most large pest control companies still use poison in their bait boxes. If you hire a pest control company, insist that they not use poison (or glue traps), or switch to a company using ethical methods. Demanding poison-free solutions helps reduce the market for poison. If there is a big enough market for exclusion and humane solutions, the poison will eventually stop. Remember that the poison cycle equals profits for pest control.

Finally, please do *not* use glue or sticky traps. They are **cruel and inhumane** and also catch songbirds, small owls, and other small animals who often have to be euthanized as a result (and only if they’re lucky). An animal caught on a glue trap, whether a rodent or a non-target animal, often suffers enormously and for a long period. It will eventually die of starvation, suffocation, pain, stress, and/or horrible injury from trying to escape. Several countries have banned glue traps, for good reason, but the United States still allows their use. Read more here (Humane Society of the United States) and here (Royal Society for the Prevention of Cruelty to Animals UK).

RAT POISONS NOT ONLY KILL WILDLIFE: THEY CAN ALSO WEAKEN AND SICKEN THEM.
Known "sublethal" impacts include:

- Hemorrhaging beneath the skin and extensive bruising. Internal hemorrhaging in bones, body wall, heart, and elsewhere in the body. Possible heart failure.[1]
- Hemorrhaging of the heart, liver, kidney, lung, intestines, and muscles.[2]
- Anticoagulants associated with inflammatory response and immune suppression in bobcats.[3]
- Anticoagulants associated with multiple system effects in bobcats.[4]
- Multiple AR exposure events associated with notoedric mange.[5]
- Barn owl clutch size, brood size, fledging success, and nest box occupancy lower in fields treated with anticoagulants.[6]
- Increased vulnerability to other causes of death such as vehicular collisions and predation.[7]
- Coyotes exposed to multiple FGARs and with high FGAR residues tended to be in poorer body condition.[8]
- Chronic anemia, making animals more susceptible to diseases, including mange, and other stressors. [9]
- Reproductive impacts. Female sheep exposed to anticoagulants had more aborted or stillborn lambs (up to 50%); male sheep had lower sperm motility.[10]
- Decreased food intake[11] and decreased body weight.[12]
- Neonatal transfer to young kits. Decreased resilience to environmental stressors.[13] Fetuses more susceptible to brodifacoum toxicity than adults.[14]

- Increased parasite and pathogen burdens.[15]
- Shorter wings, tails, bones, bills, and birth defects.[16]
- Rodents poisoned by anticoagulants are more likely to be eaten by predators.[17]
- Raptors may preferentially prey upon sickened rodents: The energetically beneficial behavior of favoring substandard prey may increase raptor encounters with rodenticide exposed animals if prey vulnerability has resulted from poisoning.[18]
- Exposure to brodifacoum may have prolonged effects that increase toxicity of subsequent AR exposure.[19]
- Bromadiolone and chlorophacinone residues from secondary poisoning can be transferred to the eggs of *T. alba.* [20]
- Increased stress and reduced body condition.[21]

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JOURNAL ARTICLE

Brodifacoum Toxicity in American Kestrels (*Falco sparverius*) with Evidence of Increased Hazard on Subsequent Anticoagulant Rodenticide Exposure

Barnett A. Rattner ✉, Steven F. Volker, Julia S. Lankton, Thomas G. Bean, Rebecca S. Lazarus, Katherine E. Horak

Environmental Toxicology and Chemistry, Volume 39, Issue 2, 1 February 2020, Pages 468–481, https://doi.org/10.1002/etc.4629

Published: 09 November 2019 **Article history ▾**

Abstract

A seminal question in ecotoxicology is the extent to which contaminant exposure evokes prolonged effects on physiological function and fitness. A series of studies were undertaken with American kestrels ingesting environmentally realistic concentrations of the second-generation anticoagulant rodenticide (SGAR) brodifacoum. Kestrels fed brodifacoum at 0.3, 1.0, or 3.0 µg/g diet wet weight for 7 d exhibited dose-dependent hemorrhage, histopathological lesions, and coagulopathy (prolonged prothrombin and Russell's viper venom times). Following termination of a 7-d exposure to 0.5 µg brodifacoum/g diet, prolonged blood clotting time returned to baseline values within 1 wk, but brodifacoum residues in liver and kidney persisted during the 28-d recovery period (terminal half-life estimates >50 d). To examine the hazard of sequential anticoagulant rodenticide (AR) exposure, kestrels were exposed to either the first-generation AR chlorophacinone (1.5 µg/g diet) or the SGAR brodifacoum (0.5 µg/g diet) for 7 d and, following a recovery period, challenged with a low dose of chlorophacinone (0.75 µg/g diet) for 7 d. In brodifacoum-exposed kestrels, the challenge exposure clearly prolonged prothrombin time compared to naive controls and kestrels previously exposed to chlorophacinone. These data provide evidence that the SGAR

brodifacoum may have prolonged effects that increase the toxicity of subsequent AR exposure. Because free-ranging predatory and scavenging wildlife are often repeatedly exposed to ARs, such protracted toxicological effects need to be considered in hazard and risk assessments. *Environ Toxicol Chem* 2020;39:468–481. © 2020 SETAC

Keywords: Wildlife toxicology, Toxicokinetics, Rodenticide, Biomarkers, Species extrapolation

Issue Section: Hazard/Risk Assessment

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ORIGINAL ARTICLE

Rodenticide contamination of cormorants and mergansers feeding on wild fish

Julia Regnery[1] · Hannah Schmieg[2] · Hannah Schrader[2] · Olaf Zinke[3] · Friederike Gethöffer[4] · Sarah-Alica Dahl[5] · Mario Schaffer[6] · Julia Bachtin[1] · Christel Möhlenkamp[1] · Anton Friesen[7]

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Abstract

Exposure of wildlife to anticoagulant rodenticides from sewer baiting and bait application is poorly understood. We analyzed residues of eight anticoagulant rodenticides in liver samples of 96 great cormorants, 29 common mergansers, various fish species, and coypu, in different German regions. Results show that hepatic residues of anticoagulant rodenticides were found in almost half of the investigated cormorants and mergansers due to the uptake of contaminated fish from effluent-receiving surface waters. By contrast, exposure of coypu to rodenticides via aquatic emissions was not observed. The maximum total hepatic anticoagulant rodenticide concentration measured in waterfowl specimens was 35 ng per g based on liver wet weight. Second-generation anticoagulant rodenticide active ingredients brodifacoum, difenacoum, and bromadiolone were detected almost exclusively, reflecting their estimated market share in Germany and their continuing release into the aquatic compartment. Overall, our findings reveal that second-generation anticoagulant rodenticides accumulating in wild fish are transferred to piscivorous predators via the aquatic food chain.

Keywords Biocides · Bioaccumulation · Biomonitoring · Persistence · Secondary poisoning

Introduction

Exposure of wildlife to anticoagulant rodenticides via the terrestrial food web is a well-known and documented environmental issue (van den Brink et al. 2018). Less documented, however, are anticoagulant rodenticide emissions to the aquatic environment and the likely transfer of persistent, bioaccumulative, and toxic second-generation anticoagulants such as brodifacoum along the aquatic food chain (Regnery et al. 2019a, 2020). Two recent studies from Germany (Regnery et al. 2024) and Pennsylvania, North America (Facka et al. 2024) clearly reinforced the relevance of previously neglected aquatic exposure pathways (Lemarchand et al. 2014). Both studies frequently detected residues of anticoagulant rodenticides in primarily piscivorous mammalian predators, Eurasian otter (*Lutra lutra*) and river otter (*Lontra canadensis*), despite the nowadays strictly regulated sale, supply, and use of rodenticides (Facka et al. 2024; Regnery et al. 2024).

As transfer and fate of anticoagulant rodenticides in the aquatic food web are not yet fully disclosed, our biomonitoring study aimed at providing further experimental evidence concerning the exposure of piscivorous predators to

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second-generation anticoagulant rodenticides via their prey in densely inhabited landscapes, such as Germany. Hence, we analyzed liver samples of 125 specimens of two exclusively piscivorous avian predators, great cormorant (*Phalacrocorax carbo*) and common merganser (*Mergus merganser*), as well as 41 liver samples of various freshwater fish species from different German regions (Bavaria, Rhineland-Palatinate, Saxony, Lower Saxony) regarding residues of all eight active ingredients used in biocidal anticoagulant rodenticides in Germany. Moreover, liver samples of 42 specimens of a semi-aquatic living, mammalian herbivore (coypu (*Myocastor coypus*)) from Lower Saxony, a region with previously documented rodenticide burden in otters (Regnery et al. 2024), were analyzed to compare their risk of exposure versus that of piscivores. We hypothesized that exposure of aquatic top predators to anticoagulant rodenticides is diet-driven, and coypu, unlike cormorants and mergansers, are thus less likely to be exposed. Chemical analyses were accompanied by post-mortem examinations of cormorant and coypu carcasses.

Experimental

Piscivorous waterfowl

The randomly investigated 96 great cormorants (*P. carbo*) from southern (Bavaria, $n = 50$), western (Rhineland-Palatinate, $n = 21$), north-western (Lower Saxony, $n = 1$), and eastern (Saxony, $n = 24$) parts of Germany (Fig. 1) belonged to the continental subspecies *P. carbo sinensis*. All cormorants had been shot near surface waters for nature conservation reasons based on state-specific species protection exception regulations between 2020 and 2023 (outside breeding season) and their carcasses were provided for post-mortem examination. In Germany, *P. carbo sinensis* inhabits the coastal areas as well as inland surface waters, with breeding occurrences in suitable habitats. Outside breeding season, encountered individuals can be sedentary birds, partial migrants, or migratory birds, respectively, as the Baltic Sea population generally migrates overland and winters from southern Germany to North Africa. Due to their vast foraging grounds and high mobility (cormorants may roam widely during the day and visit multiple feeding waters), exact origins of their fish prey cannot be determined with certainty.

Twenty-nine liver tissue samples of common mergansers (*M. merganser*) were received from an on-going

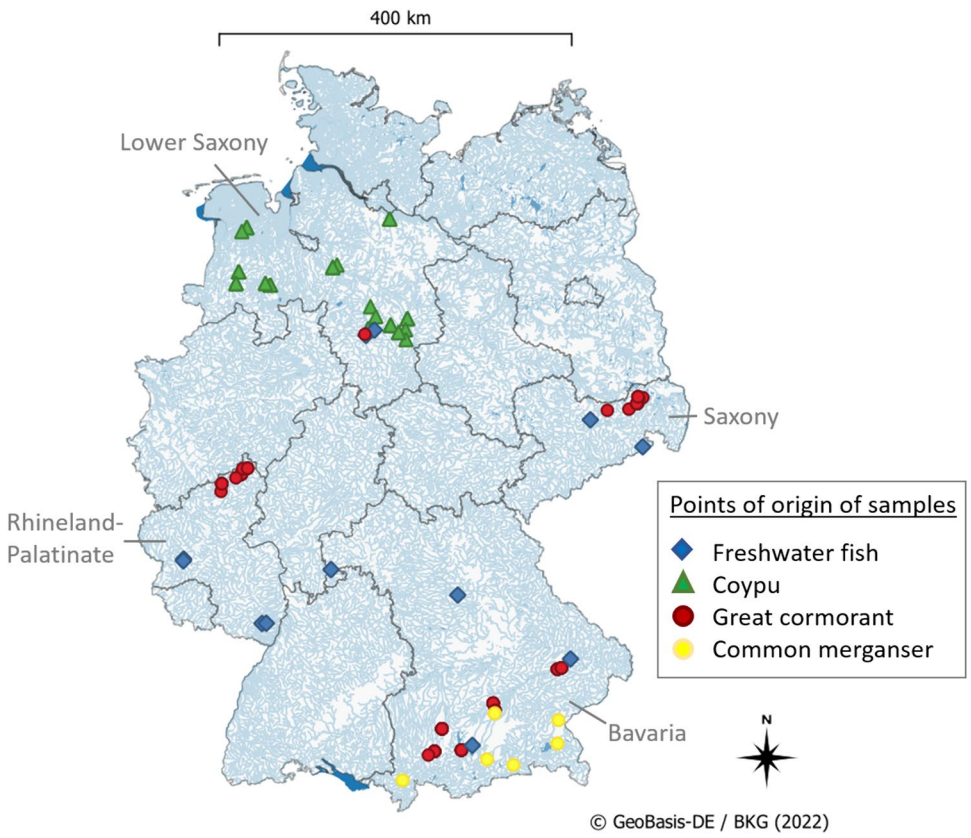

Fig. 1 Location of 208 samples of fish, coypu, cormorant and merganser analyzed for liver tissue. Please note that specimens originating from the exact same location are not illustrated by individual symbols

research project (FKZ A/20/03) about deterrence measures for nature conservation by Technical University of Munich, Wildlife Biology and Management Unit in collaboration with the Bavarian State Research Center for Agriculture, Institute for Fisheries. Adult birds had been culled at 6 selected stream sites in southern Germany (Fig. 1) in early spring 2023 (prior to the start of breeding season). In southern Bavaria, the common merganser lives as a sedentary bird year-round, with additional individuals passing through during winter months. Similar to cormorants, their prey consists primarily of small fish the size of 10–15 cm, which they hunt by diving in open surface waters. Thus, their foraging grounds generally overlap with those of great cormorants.

Freshwater fish

Freshwater fish sampling sites (Fig. 1) were in the broader vicinity of potential foraging grounds of analyzed cormorants and mergansers and included two streams each in Lower Saxony (Innerste, Leine) and Rhineland-Palatinate (Moselle, Queich), one stream in Saxony (Elbe), as well as one lake (Starnberger See) and three streams (Main, Isar, Pegnitz) in Bavaria. Individual ($n = 35$) and pooled ($n = 6$) liver tissue samples of species from different trophic levels such as common nase (*Chondrostoma nasus*), bleak (*Alburnus alburnus*), roach (*Rutilus rutilus*), chub (*Squalius cephalus*), brown trout (*Salmo trutta* f. *fario*), perch (*Perca fluviatilis*), pike (*Esox lucius*), pike-perch (*Sander lucioperca*), and European catfish (*Silurus glanis*) were kindly provided by the Bavarian Environment Agency, the Lower Saxony Water Management, Coastal and Nature Protection Agency, the Structural and Approval Directorate South (Upper Fisheries Authority) Rhineland-Palatinate, and the River Basin Community Elbe. The majority of liver tissue samples originated from fish caught between 2019 and 2023 during European Water Framework Directive biota monitoring campaigns.

Semi-aquatic living rodent

M. coypus, a semi-aquatic, invasive alien species with a plant-based diet, is classified as huntable game in most German federal states. A total of 42 coypu carcasses were obtained for post-mortem investigations from 17 different surface water locations in Lower Saxony (Fig. 1), at which coypu had been culled by hunters within the exercise of hunting rights between November 2020 and April 2021. Coypu are mainly nocturnal and crepuscular, respectively, and tend to stay along banksides during foraging.

Post-mortem investigation

Great cormorant carcasses from Saxony were examined according to routine procedures at the Museum of the Westlausitz Kamenz, whereas cormorant carcasses from Rhineland-Palatinate and Bavaria were handled at the Bavarian Environment Agency. Post-mortem examination of coypu carcasses and the single great cormorant from Lower Saxony was conducted at the Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Foundation. Recorded parameters for both species included biometric data, sex, estimated age, and nutrition status. For several specimens, the stomach content was also exemplarily recorded. Freezing of the carcasses prior to examination had prevented adequate blood sampling to screen for acute anticoagulant rodenticide poisoning characterized by coagulopathy. All sampled liver tissue was immediately frozen and shipped express on ice to the Federal Institute of Hydrology laboratory for chemical analyses.

Analytical methods and data analysis

Established analytical methods (Regnery et al. 2019b, 2024) were used for the quantitative chemical analysis of one pharmaceutical (phenprocoumon) and 8 biocidal (brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen, coumatetralyl, chlorophacinone, warfarin) anticoagulant active ingredients in liver tissue samples by liquid chromatography–tandem mass spectrometry. Method performance parameters for investigated species such as average recovery rates, method quantification limits, and estimated expanded measurement uncertainties are summarized in Tables S1–S3 (Supplementary Material) or already provided elsewhere (Regnery et al. 2019b, 2024). All reported analyte concentrations in liver tissue are based on wet weight. In addition, total hepatic lipid content of selected specimens was determined as described in Regnery et al. (2019b). Whenever total anticoagulant rodenticide concentrations are discussed in the following, residues of biocidal anticoagulants had been summed for each specimen, i.e., at least one of eight active ingredients detected above its respective method quantification limit, zero assigned for values below these limits. OriginPro, version 2021b (OriginLab Corporation, Northampton, MA, USA) was used for graphing and nonparametric Kruskal–Wallis analysis. Statistical difference was considered significant when $p < 0.05$.

Results and discussion

Age, sex, and body condition of examined specimens

The majority of investigated cormorants (i.e., 44 juveniles, 52 adults) was well nourished. Their determined total hepatic lipid contents were in the range of 2.7 ± 1.3% (in mergansers 5.0 ± 0.5%). The average body weights of female ($n = 34$) and male ($n = 61$) cormorants were 2182 ± 336 g and 2570 ± 321 g, respectively. Almost all cormorants had numerous nematodes in their gastrointestinal tracts. While stomach contents mainly consisted of small fish the size of 7–15 cm total length, a few larger fish up to 26 cm total length were also found. Identified ingested fish species were carp (*Cyprinus carpio*), chub, roach, and perch. The health condition of investigated coypu was predominantly good. Approximately two thirds were well nourished and observed stomach contents were considered typical for this herbivorous species. The average body weight of investigated coypu (i.e., 16 juveniles, 26 adults) was 3732 ± 1591 g for females ($n = 18$) and 4651 ± 1798 g for males ($n = 23$). Determined total hepatic lipid contents were in the range of 3.2 ± 0.6%.

Measured hepatic second-generation anticoagulant rodenticide residues

Overall, 46 out of 96 cormorants (47.9%) from all four regions exhibited quantifiable anticoagulant rodenticide residues in their livers, mostly from 1–2 second-generation anticoagulant rodenticide active ingredients with a maximum total anticoagulant rodenticide burden of 35.1 ng/g (Fig. 2). Concentrations measured in males and females indicated no statistical difference (Kruskal–Wallis test, $H(1) = 0.342$, $p = 0.559$). Brodifacoum was detected in 39 (max. concentration of 27.6 ng/g), difenacoum in 23 (max. 7.5 ng/g), and bromadiolone in 3 (max. 2.3 ng/g) specimens, respectively. Coumatetralyl was solely detected in one cormorant liver tissue sample at very low concentration (0.18 ng/g), corroborating the lesser bioaccumulation potential of first-generation

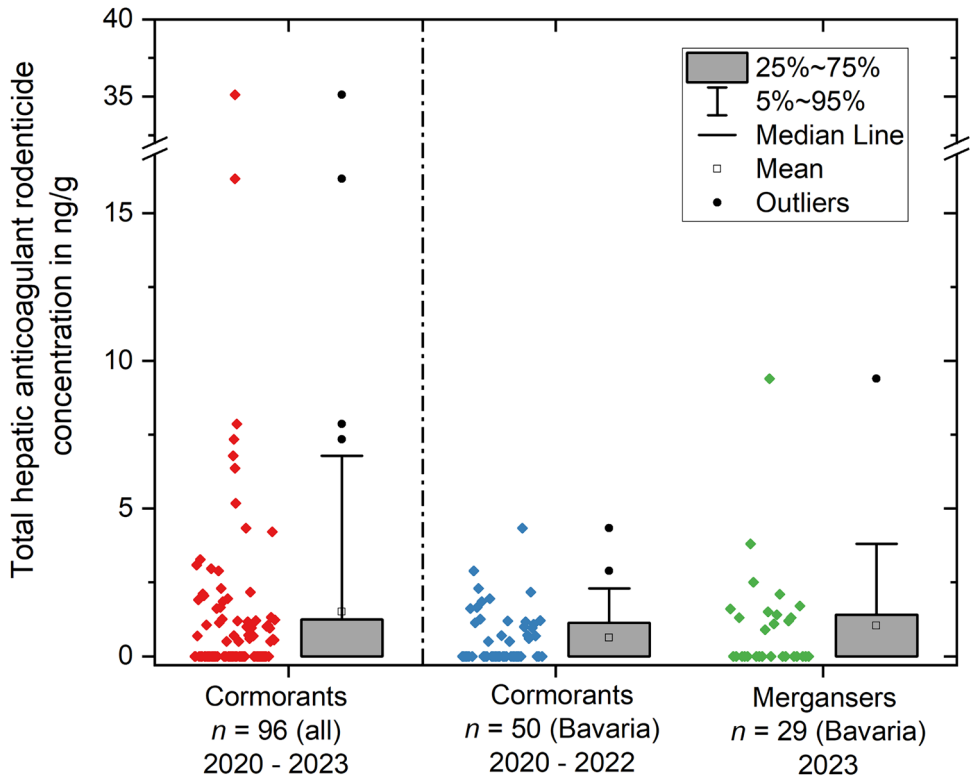

Fig. 2 Box plots of measured total anticoagulant rodenticide residue concentrations in liver tissue samples of investigated cormorants and mergansers from different German regions that had been shot near surface waters between 2020 and 2023. Residues of detected biocidal anticoagulants had been summed for each specimen, zero was assigned for values below the respective method quantification limits. Overall, 46 out of 96 cormorants (47.9%) and 13 out of 29 mergansers (44.8%) exhibited quantifiable anticoagulant rodenticide residues in their livers, mostly from 1 to 2 second-generation anticoagulant rodenticide active ingredients with a maximum total anticoagulant rodenticide burden of 35.1 ng/g based on wet weight. Rodenticide residue concentrations were not significantly different among groups, i.e., among all cormorants and cormorants and mergansers from Bavaria (Kruskal–Wallis test, $H(2) = 0.773$, $p = 0.679$)

anticoagulant rodenticides. In good agreement with findings from cormorants shot near Bavarian surface waters (Fig. 2), hepatic anticoagulant rodenticide residues were also detected in 13 out of 29 mergansers (44.8%), mostly from one second-generation active ingredient. Brodifacoum was detected in 12 specimens (max. concentration of 9.4 ng/g), bromadiolone in 2 (max. 1.6 ng/g), and difenacoum in one (0.5 ng/g), respectively. Residue levels of brodifacoum, difenacoum, and bromadiolone were not related to hepatic total lipid contents. Flocoumafen, difethialone, chlorophacinone, warfarin, and the pharmaceutical anticoagulant phenprocoumon were not detected above their respective method quantification limits in the analyzed waterfowl liver samples.

In contrast, solely one adult coypu exhibited elevated residues of 135.4 ng/g difenacoum in its liver, together with traces of a second active ingredient (1.1 ng/g brodifacoum). It should be emphasized that none of the biocidal and pharmaceutical anticoagulants were detected in any of the other 41 analyzed coypu. Thereof were 3 specimens that had been culled at the same location as the exposed one. In wild freshwater fish, measured total hepatic anticoagulant rodenticide concentrations (Fig. 3) matched previous records of rodenticides in fish from these effluent-receiving streams, e.g., Main, Isar (Regnery et al. 2019b), Elbe (Kotthoff et al. 2019), Moselle, Queich (Regnery et al. 2020), illustrating the continued emission of rodenticides from sewer baiting and outdoor surface baiting into the aquatic compartment. Their absence in fish from Starnberger See, an effluent-free lake, was also in good agreement with previous records (Regnery et al. 2019b). Highest total hepatic second-generation anticoagulant rodenticide levels in fish (mainly brodifacoum) of 74.5 ng/g (roach, 26 cm total length) and 95.6 ng/g (chub, 30.5 cm total length) were detected at two stream sites in Rhineland-Palatinate (Queich) and Lower Saxony (Innerste), respectively. At both sites, sewer baiting measures using baits deployed by wire in combined sewer systems had been carried out shortly before fish sampling campaigns, according to released public press communications.

Diet-driven exposure risk

As mentioned earlier, the exact origins of the waterfowl's ingested fish prey, and thus second-generation rodenticide residues, were unknown. Four cormorant individuals shot at surface waters in Bavaria had been tagged in Latvia, Finland, Switzerland, and Northern Germany, respectively. The limited and unforeseeable availability of biological tissue samples from protected species did not allow for strategic collection of corresponding predator and prey samples to ascertain full spatial and temporal overlap. Moreover, the prey composition of cormorants usually depends on what fish can be caught at all, or with as little effort as possible, rather than a strong preference for certain fish species (Keller 1998). Yet, the continuous presence of hepatic second-generation anticoagulant rodenticides in fish from effluent-receiving streams in the vicinity of foraging grounds of analyzed cormorants

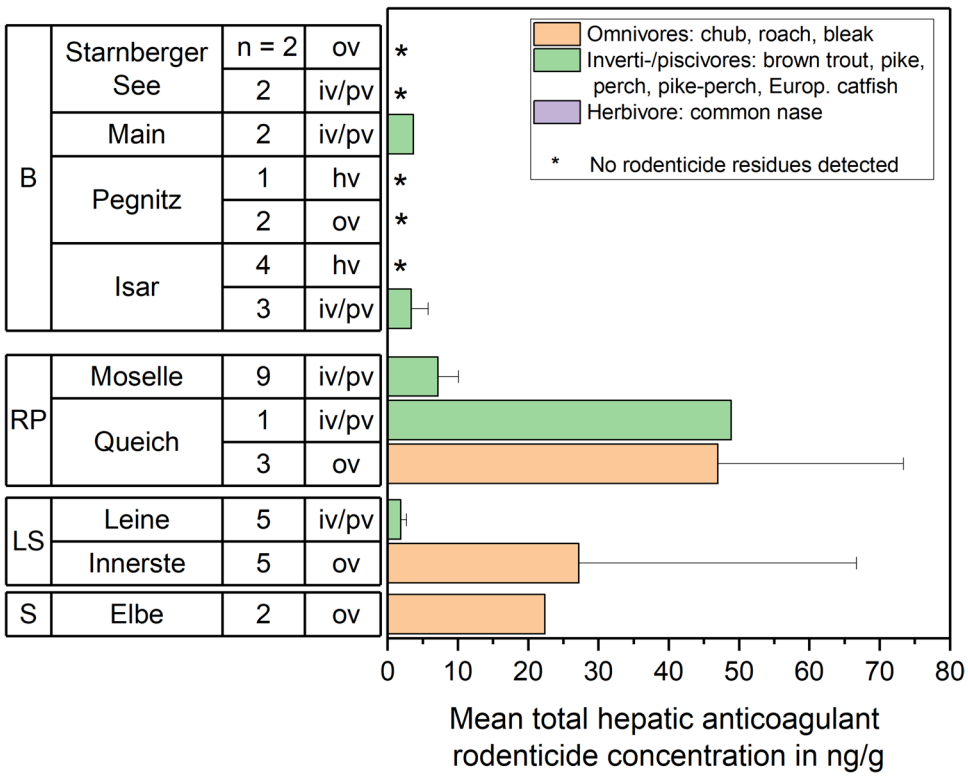

Fig. 3 Mean total anticoagulant rodenticide residue concentrations in liver tissue samples ($n = 41$) of different herbivorous (hv), omnivorous (ov), and inverti-/piscivorous (iv/pv) fish species from multiple surface water sampling sites located in Bavaria (B), Rhineland-Palatinate (RP), Lower Saxony (LS), and Saxony (S). Concentrations of detected biocidal anticoagulants, based on liver wet weight, had been summed for each specimen. Specimens were grouped by feeding-type, which presumably is a determining factor in second-generation anticoagulant rodenticide uptake. Where applicable, the relative standard deviation of mean values is shown. Highest total hepatic second-generation anticoagulant rodenticide levels in fish were observed at two stream sites (Queich, Innerste) with nearby sewer baiting

and mergansers demonstrates that exposure of piscivorous avian predators occurs via their fish prey. Residue levels in the analyzed waterfowl also clearly reflected current use patterns and the market dominance of brodifacoum, difenacoum, and bromadiolone containing biocidal products in Germany (Regnery et al. 2024). Another unequivocal indication was the absence of low-level anticoagulant rodenticide residues in coypu from Lower Saxony, a region previously known for pronounced anticoagulant rodenticide use and thus frequent detection in otters (Regnery et al. 2024). As pointed out in a recent review, including species from a diversity of trophic levels during biomonitoring is very helpful to comprehend exposure pathways (Keating et al. 2024). Primary exposure to difenacoum-containing bait was deemed most plausible to explain the elevated concentration detected in one adult coypu. Although their body size should prevent them from directly accessing tamper-resistant bait station, loose grain bait may be attractive for coypu when accessible. For instance, when baits are spilled from bait stations deployed near banks or deliberately offered.

Primary exposure of cormorants and mergansers to rodenticide bait, on the other hand, is considered extremely unlikely. The seemingly low hepatic rodenticide levels of investigated piscivorous waterfowl (Fig. 2) compared to reported secondary poisoning levels in predatory wildlife of the terrestrial food web (van den Brink et al. 2018) can most likely be explained by the absence of residues in fish from fish rearing ponds and surface waters without wastewater-borne rodenticide emissions (Regnery et al. 2019b; Kotthoff et al. 2019) that are frequently visited by cormorants during foraging (Keller 1998). Additional factors concerning piscivorous avian predators, such as the regurgitation of food if alarmed and a higher body temperature compared to mammals, may play a role too in terms of bioaccumulation and biotransformation (Kuo et al. 2022). The absence of second-generation anticoagulant rodenticides in 5 liver samples of common nase, a predominantly herbivorous fish species, also suggests that the foraging strategy is a determining factor in second-generation anticoagulant rodenticide uptake in the aquatic food web, e.g., such as the diversity and complexity of diets. Other fish caught at the same time at the Isar sampling site exhibited hepatic rodenticide residues in comparison (Fig. 3). However, more research (and data) will be required for a sound statistical assessment of such complex food web relationships.

Conclusion

Extensive knowledge and understanding of actual exposure pathways of biocidal anticoagulant rodenticides is essential to improve environmental exposure and risk assessments, and consequentially risk mitigation measures for the aquatic environment. Our biomonitoring study demonstrated that piscivorous avian predators in anthropogenically influenced landscapes are exposed to second-generation anticoagulant rodenticides via their fish prey. Transfer of second-generation active ingredients along the aquatic food chain was thus confirmed. Without doubt, future improvements of regulatory measures concerning biocides will be required to mitigate the yet unknown consequences for aquatic wildlife from the nowadays almost exclusive application of second-generation anticoagulant rodenticides during chemical rodent control.

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Author contributions Julia Regnery: Conceptualization, Validation, Formal analysis, Investigation, Writing—original draft, Funding acquisition; Hannah Schmieg: Data curation, Investigation, Writing—review & editing; Hannah Schrader: Data curation, Investigation, Writing—review & editing; Olaf Zinke: Data curation, Writing—review & editing; Friederike Gethöffer: Data curation, Investigation, Writing—review & editing; Sarah-Alica Dahl: Data curation, Writing—review & editing; Mario Schaffer: Data curation, Writing—review & editing; Julia Bachtin: Investigation, Writing—review & editing; Christel Möhlenkamp: Investigation, Writing—review & editing; Anton Friesen: Conceptualization, Writing—review & editing, Supervision.

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Data availability Data will be made available on request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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SAFE RODENT CONTROL: REAL-LIFE SOLUTIONS

Got Rats or Mice?

Don't turn to toxic, dangerous rodenticides to get rid of rodents. Instead, use exclusion and sanitation tactics to make sure they never enter your home or business in the first place. Sealing entry points helps prevent rodents from accessing buildings, and eliminating food, water, and shelter heads off a full-scale invasion.

Keep reading to learn about safe, cost-effective ways to thwart rodent infestations without using poisons that could hurt your family, your pets, and rare wildlife.

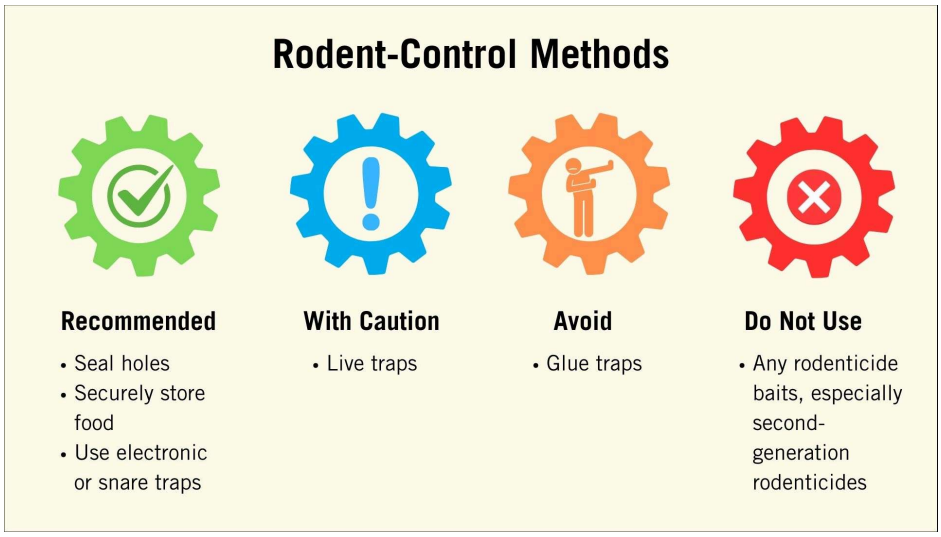
(The tips on this page are specifically for rats and mice, but we also have tons of ideas for rodenticide-free gopher, mole, and vole control.)

The Basics of Rat and Mouse Control

Rats and mice aren't just a nuisance — they can also damage property and spread diseases. So if you find signs of rodents, you want them gone forever, right? Poisons won't help with that —you'll have to keep poisoning rodents (and disposing of their bodies) over and over.

Instead, follow our three guiding principles for maintaining a rodent-free, rodenticide-free home: *prevent, identify, treat.* Many pest-control companies can help, but make sure they avoid rodenticides.

Prevent	Identify	Treat
Seal entry points to prevent rodents from entering your home or business. Be sure to use 1/4" x 1/4" metal mesh, steel wool, or insulating foam with a bittering agent to seal off existing entry points.	Look for signs of rats and mice: rodent droppings around food, in kitchen corners, inside cabinets, and under sinks; smudges and dark marks near entry points or nests; and chew or bite marks on food, wood, cardboard, or plastic.	Install rodent fertility-control bait stations. Remove existing rodents with snap or electronic traps.
Remove rodent attractants like consumables and shelter by ensuring that food and water are secure and surroundings are clean.	Also look for nesting material, such as shredded paper or fabric.	Install barn owl nesting boxes to draw owls who'll control rodents naturally.



Prevent

Eliminate Rodent Attractants and Entry Points

To keep rats and mice away permanently, prevent access to the building by sealing all possible entry points. It's equally important to eliminate rodent attractions like food and water by keeping food tightly sealed and repairing leaks. Periodically do a sweep of the exterior and interior of your building(s) to make sure your property isn't appealing for rodents.

Outdoor recommendations:

- Don't plant ivy — it provides shelter and harbors snails and slugs, a food source for rodents. Ivy on walls can form "rat ladders" to windows, attics, and other interior spaces.
- Keep compost piles as far away from structures as possible and grass cut to no more than 2 inches tall.
- Don't leave pet food or water out overnight.
- Maintain at least a 2-foot space between bushes, shrubs, fences, and buildings and remove tree limbs within 3 feet of a structure or roof.
- Avoid bird feeders, since they can also provide food for rodents.
- Keep outdoor grills and cooking areas clean.
- Keep firewood off the ground and as far away from structures as possible to mitigate shelter opportunities.
- Use trash bins that close securely to keep out rodents. If a bin is cracked or missing a lid, contact the Department of Sanitation for a replacement.

Indoor recommendations:

- Eliminate food and water sources:
  - Food in unsealed containers, like opened bags of chips, rice, cereal, crackers, flour, and other nonperishables
  - Pet food left out overnight or in an open bag instead of a secure container
  - Fruits or vegetables in bowls left outside of refrigerator
  - Leaky pipes or faucets
  - Open trash and compost containers
- Opt for garbage bins and compost containers with tops that seal tightly.
- Rinse food and beverage containers before discarding or recycling them.
- Clean your garbage and recycling bins frequently.
- Keep stovetops clean and free of food scraps.
- Maintain attic, crawlspaces, and cabinets near sinks clean and free of moisture.
- Declutter your home of papers, fabric, and other materials that rodents use for nesting.
- Use steel wool, caulk, foam with a bittering agent, or 1/4" x 1/4" metal mesh to seal these common entry points:
  - Holes near cabinets, closets, or doors leading outside or to crawlspaces and attics.
  - Holes around sink or appliance pipes
  - Cracked foundations in the basement or unscreened ventilation holes in the attic, especially in older structures
  - Gaps or cracks in baseboards
  - Holes in and around windows and doors
  - Missing screens in vents or crawlspaces under buildings

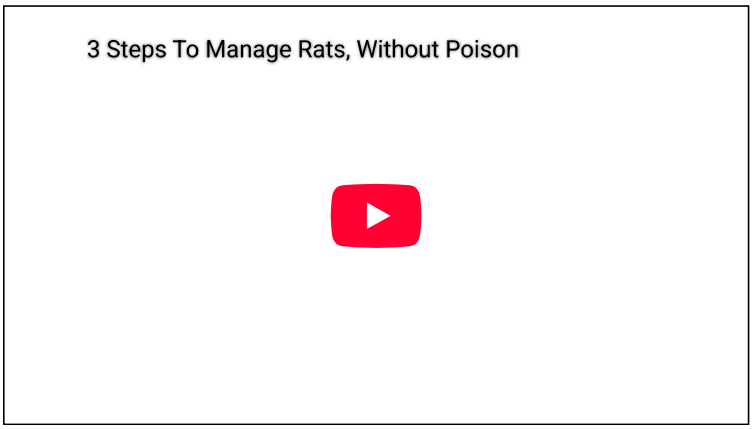
For even more rodent-deterrent tips, visit Raptors Are the Solution's Got Rats? webpage and Humane Pest Control's In Buildings webpage.

Promote Natural Predators

What's more of a deterrent to rodents than getting eaten?

Wild predators like snakes, hawks, and owls can help control rodent populations by gobbling up rats and mice before they get into your home. Barn owls are especially efficient hunters — a single family of barn owls can eat as many as 3,000 mice every year. To encourage barn owls to nest and hunt in your area, consider installing one or more nesting boxes. Strategic placement of nesting boxes combined with the use of traps and other preventative measures will go a long way toward managing your rodent problems.

For more information on installing *and* maintaining nesting boxes, visit the Hungry Owl Project. It's crucial to note that the Hungry Owl Project strongly urges you to use norodent poisons *at all*, either indoors or outdoors, while encouraging owls to spend time on your property. Rodent poisons could kill an owl who eats a poisoned rodent.



Identify

Look Out for Signs of Rodents

It might take some practice to master prevention methods, and of course sometimes new rodent access points and attractants will pop up before you notice them. Or maybe rodents got into your home before you discovered this webpage.

If any clever rats or mice have already invaded or ever sidestep your deterrent efforts, you'll know they've arrived if you're observant.

Stay alert to identify rodent stowaways by these telltale signs:

- Droppings, especially near a food source
- Shredded fabric or paper, which rodents use to nest
- Gnaw marks on food packaging or the building structure
- Scratching noises coming from the walls or ceiling, especially at night
- A musty, stale odor
- Greasy marks where rats have rubbed against a wall or doorway
- Unusual pet behavior

Treat

Decide How to Get Rid of Any Existing Rodents

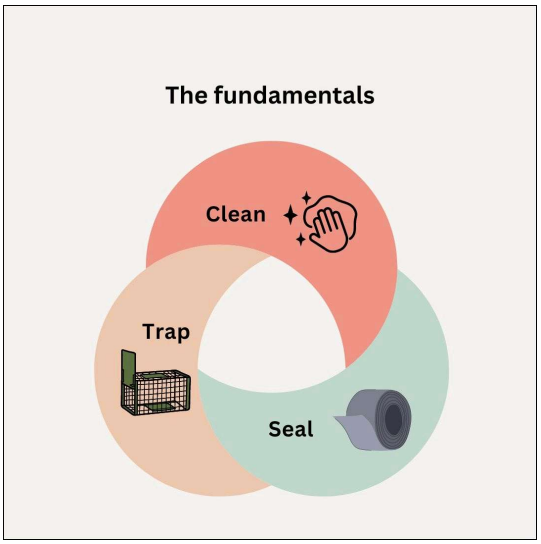
If you do see signs of rats or mice, we recommend a combination of continuing preventative measures and cautiously using the right kinds of traps or other treatment options, like hiring a professional.

Review all your options before deciding on a treatment plan. If you decide to work with a pest control professional, be sure the company is certified by Ecowise or GreenShield and familiar with integrated pest-management techniques. Integrated pest management is an environmentally sensitive approach focusing on long-term solutions by relying on common-sense practices and current, comprehensive information on pests' lifecycles and how they interact with the environment.

Consider Fertility Control

Rodent fertility control is an effective method to reduce rodent infestations in an environmentally friendly way. In a 2022 study examining the effectiveness of rodent-fertility products in reducing roof rat populations on a California poultry farm, rat activity was reduced by 94% with the incorporation of fertility control into existing integrated pest management. Place rodent birth control stations near locations with rodent infestations to eliminate the prolific reproductive cycle of rodents, which leads to infestations.

Even when using traps, remember: Using a multi-tactic approach to managing rodents — including fertility control — decreases the risk of dealing with future infestations.



Types of Traps

Traps are far from a perfect solution. Still, they're much better than rodenticides. Besides eliminating the dire threats that rodenticides pose to people, pets, and wildlife (including natural predators), using traps instead of poisons gives you clear confirmation of captured rodents, letting you better gauge treatment effectiveness. Traps also let you dispose of rodents immediately instead of dealing with rotting poisoned rodents who may die in out-of-reach places. For a guide on how to select and place traps, watch this video by the New York State Integrated Pest Management Program and check out our overview below.

Trap	Description
Snap trap	This is the oldest type of trap and uses a spring-loaded bar to kill a rodent on contact. Many modern snap traps prevent risk to children and pets by enclosing the device in a plastic box — which also hide the dead rodent, make for easier rodent disposal, and can be reused. Watch this instructional video on how to safely set a snap trap.
Electronic trap	This battery-powered trap delivers an electric shock that kills rodents quickly. It's a newer type of trap, and models are available for both rats and mice.
Live animal trap	This catch-and-release system that avoids killing a rat or mouse. Just note that some states prohibit releasing rodents into the wild, and the CDC warns that captured rats or mice might urinate, increasing the risk of disease spread.
Multiple-catch live mouse trap	This is a catch-and-release system that allows for capture of multiple mice. (See our warnings about live animal trap above.)
Glue trap	We *don't* recommend glue traps because the adhesive plate they use to capture rodents can also trap birds, baby animals, lizards, and even pets. Plus, these traps cause rodents undue suffering — and while they're waiting to die, they may also urinate and increase the risk of spreading disease.

Trapping Rodents Safely

It's still crucial to use traps safely and then properly dispose of dead rodents to reduce the risk of getting sick or spreading disease.

Trap-use tips:

- Always read and follow the label instructions on traps.
- Place traps out of children's and pets' reach or inside safety enclosure boxes.

Tips for rodent and nesting-material disposal:

- Use gloves when disposing of dead rodents, nests, or nesting material.
- Spray the dead rodent or nesting material with a disinfectant solution and allow it to soak for 5 minutes before disposing of the rodent or material in a secure plastic bag.
- Spray and wipe up the area surrounding the dead rodent or nesting material with a disinfectant.
- Place the plastic bag with the rodent or nesting material into another plastic bag, along with any wipes or rags you used to sanitize the area.
- Be sure to wash your hands thoroughly with soap and water when you're done.

Needs more tips? Check out these step-by-step tips on cleaning up after rodents — dead rodents from traps, rodent urine and droppings, and rodents in old vehicles — from the Centers for Disease Control and Prevention.

If You Use Rodenticides Despite the Consequences

Some people choose to use rodenticides despite their massive dangers and drawbacks, including their high risk of causing suffering and death in children and nontarget animals (and the fact that they'll probably leave dead rodents in your walls). If you or someone you know resort to this option, at least follow these guidelines to reduce the risks of using such potent poisons:

- Always read and follow the label instructions on the pesticide product. The label is the law, and you could be liable for any damage resulting from not following the label instructions.
- Use only products approved by the U.S. Environmental Protection Agency that are sold and used with tamper-resistant bait stations to help reduce poisonings of children, pets, and wildlife. Here's a list of EPA-approved rodenticide bait station products.
- Indoors, only put rodenticide bait stations in places completely inaccessible to children and pets: inside walls, under heavy appliances, or in enclosed crawlspaces.
- To protect wildlife, don't use any rodenticides containing anticoagulants (brodifacoum, bromadiolone, difenacoum, difethialone, diphacinone, warfarin, and chlorophacinone) as active ingredients.
- Once all signs of rodents are gone, remove bait stations promptly by placing them in a secure plastic bag.

More Resources

- Raptors Are the Solution: Got Rats?
- Northwest Center for Alternatives to Pesticides: Controlling Rats Without Poisons
- Poison-Free Malibu: Earth-friendly management techniques so people can make informed decisions about pesticides usage
- University of Florida's IFAS Extension
- U.S. Environmental Protection Agency: Identify and Prevent Rodent Infestations

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RELATED CAMPAIGNS
Safe Rodent Control
Carnivore Conservation
Cyanide Bombs
Environmental Health
Pesticides Reduction

MEDIA
Press releases
Search our newsroom

SPECIES
Alameda whipsnake
Bald eagle
California condor
California mountain lion
Desert nesting bald eagle
Fisher
Florida panther
Northern spotted owl
San Joaquin kit fox

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

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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

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

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).

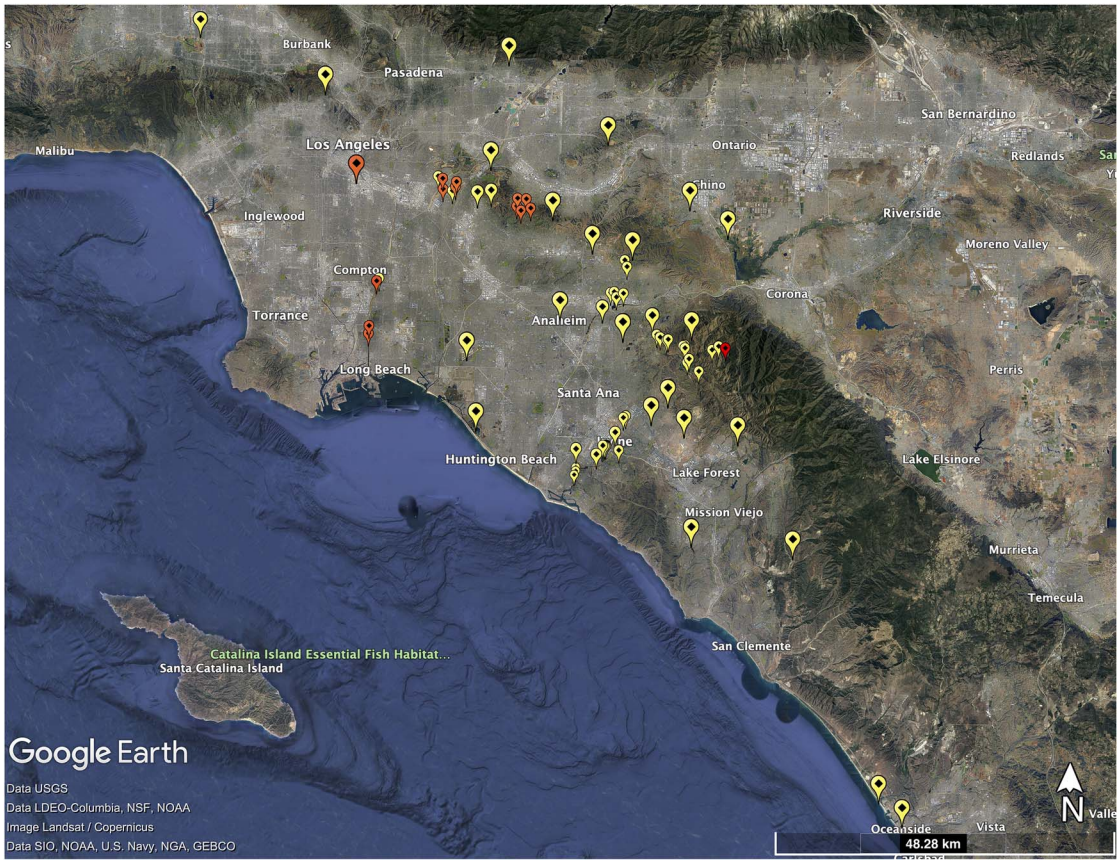

Figure 1. Visual sightings for wing-tagged southern California Turkey Vultures. Each icon in the figure corresponds to a sighting location of one of 19 different Turkey Vultures. Each icon indicates one sighting, except for a few areas in which sighting locations were identical. Larger icons indicate 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

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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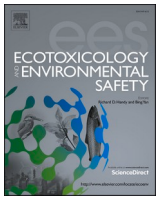

Brodifacoum causes coagulopathy, hemorrhages, and mortality in rainbow trout (*Oncorhynchus mykiss*) at environmentally relevant hepatic residue concentrations

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ABSTRACT

Widely used second-generation anticoagulant rodenticides like brodifacoum are classified as persistent, bioaccumulative, and toxic. Widespread exposure of terrestrial and avian non-target species is well-known and recently hepatic anticoagulant rodenticide residues have been detected in wild fish. However, no sufficient data exist to interpret the effects of these findings on fish health. In order to assess the potential impact of rodenticide residues on fish, we exposed rainbow trout (*Oncorhynchus mykiss*) to brodifacoum-spiked feed. In a first experiment, individually kept trout (body weight ca. 200 g) were exposed to a single dose of brodifacoum and observed for 15 days. In a second experiment, fish (body weight ca. 330 g) were kept in groups and fed every 7 or 8 days with brodifacoum-spiked feed for up to 60 days. Sampling of trout every 15 days over the 60 days period allowed monitoring of brodifacoum concentrations in serum, liver, and muscle tissue, as well as occurring effects over the course of the experiment. In both experiments, brodifacoum doses of $\geq$ 75 µg/kg body weight caused prolonged or non-measurable blood coagulation times. Disturbed hemostasis led to hemorrhages and anemia with significantly decreased albumin levels. In the 60 days-experiment, brodifacoum doses $\geq$ 100 µg/kg body weight caused additionally discoloration, apathy, and anorexia, resulting in reduced weight gain, and ultimately mortality. The delay until the onset of overt symptoms (14–17 days) highlights the importance of test duration while investigating effects of anticoagulant rodenticides in fish. The lowest hepatic brodifacoum concentration associated with effects in trout was on average 122.6 ng/g liver wet weight, which is in the range of previously reported brodifacoum residues in wild fish. These findings illustrate the risks associated with the use of anticoagulant rodenticides for freshwater fish and reinforce the need to stipulate all available and appropriate risk mitigation measures to prevent emissions at source.

1. Introduction

Since the middle of the 20th century, the mainstay of rodent control measures has been anticoagulant rodenticides (ARs) (Berny et al., 2014; Buckle and Eason, 2015), of which potent second-generation anticoagulant rodenticides (SGARs) such as brodifacoum are classified as persistent, bioaccumulative, and toxic (PBT; Regnery et al., 2019a; van den Brink et al., 2018). Unintentional poisoning of non-target wildlife occurs via direct consumption of AR-containing bait (primary poisoning), feeding of intoxicated animals or carcasses (secondary poisoning), or via environmental emissions (López-Perea and Mateo, 2018; Regnery et al., 2019a; Shore and Coeurdassier, 2018). As detailed in an extensive review by Rattner et al. (2014b), ARs inhibit the vitamin K epoxide reductase (VKOR), causing a deficiency of vitamin K. Vitamin K deficiency results in a lack of functional vitamin K dependent proteins like the clotting factors II, VII, IX, and X. After intoxication with ARs, already produced functional clotting factors are used up, which is why the onset of coagulopathy takes several days. Appearing symptoms, apart from spontaneous or trauma related hemorrhages, are anemia, pale mucosa, lethargy, and anorexia. Animals can die of blood loss or

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succumb to relatively minor hemorrhages causing localized ischemia, hypoxia, and cell death at vital sites (Rattner et al., 2014b; Rattner and Mastrota, 2018; Valchev et al., 2008). To monitor wildlife exposure to ARs, most studies focused on mammals and birds (López-Perea and Mateo, 2018; Shore and Coeurdassier, 2018). However, linking hepatic AR concentrations with lethal or sublethal effects remains challenging as toxicity varies considerably amongst species and individuals (Rattner et al., 2014b; Rattner and Mastrota, 2018; Thomas et al., 2011). In a probabilistic analysis, Thomas et al. (2011) calculated toxicity thresholds for different predatory bird species. Hepatic SGAR residues as low as 80 ng/g wet weight were associated with a 20 % chance of becoming symptomatic (Thomas et al., 2011).

In recent years, awareness regarding the presence of aquatic AR exposure pathways and associated risks emerged (Regnery et al., 2024, 2019a and references therein). SGAR residues have been frequently detected in the ng/g concentration range in liver tissue of fish caught alive (Cavanagh and Ward, 2014; Kotthoff et al., 2019; Masuda, 2014; Regnery et al., 2024, 2020, 2019b). Furthermore, the presence of VKOR and the vitamin K dependent clotting factors have been demonstrated in fish (Beato et al., 2020; Hanumanthaiah et al., 2001; Tavares-Dias and Oliveira, 2009). Yet, detailed studies on effects of SGARs on fish are scarce and have limited informative value, especially with reference to associated hepatic residue levels. It is well known that the effects of warfarin, a pharmaceutical and first-generation anticoagulant rodenticide (FGAR), on fish are comparable to mammals (e. g., Fernández et al., 2014; Granadeiro et al., 2019; Jung and Kawatsu, 1995). Reported LC_{50} (lethal concentration for 50 % of the test organisms) values for rainbow trout (*Oncorhynchus mykiss*) exposed to ARs for 96 h via the water phase range from 40 μg/L for brodifacoum to 65,000 μg/L for warfarin (eCA, 2016a, 2016b; Regnery et al., 2019a). Furthermore, Wu et al. (2023) reported sublethal effects of 90 h exposure to brodifacoum in zebrafish (*Danio rerio*) at concentrations ≥ 200 μg/L and a significant effect on survival at a concentration of 800 μg/L. Considering the low water solubility of SGARs, it seems more likely that environmental exposure of fish to SGARs occurs via dietary uptake rather than aqueous exposure (Regnery et al., 2019a). It has been assumed that carcasses of poisoned animals or terrestrial invertebrates feeding on rodenticide bait can transfer ARs to aquatic organisms (Regnery et al., 2020, 2019a and references therein). According to biomonitoring data, feeding-type appears to be a determining factor in SGAR uptake in fish (Regnery et al., 2024, 2020). One study assessed the acute toxicity of diphacinone, chlorophacinone, and brodifacoum to multiple fish species in relation to hepatic concentrations (Riegerix et al., 2020). After difficulties with oral uptake of bait, capsulated AR solution or AR-spiked feed, fish were exposed via intraperitoneal injection (IP), followed by a 72 h observation period. Beside mortality, the Russell's viper venom time of exposed fish was evaluated and was prolonged depending on the AR dose. The most toxic AR for fish in the experiment by Riegerix et al. (2020) was brodifacoum with a median lethal dose (LD_{50}) range between 36,000–96,000 μg/kg body weight (bw) and a corresponding average liver concentration of 38,100 ng/g brodifacoum based on wet weight. In another study, the whole-body brodifacoum residues of wild fish found dead after airborne bait distribution during rat eradication measures, indicating a lethal intoxication, ranged between 58–1160 ng/g wet weight, whereas other fish caught alive days afterwards exhibited whole-body brodifacoum residues up to 315 ng/g wet weight (Pitt et al., 2015). Apart from that, to our knowledge, no experimental data exists to interpret the effects of AR residues in wild fish in the context of environmentally relevant concentrations and routes of exposure.

To evaluate the potential impact of ARs on wild fish, it is key to understand the relationship of liver concentrations of the substances as observed in biomonitoring studies and the effects on fish health associated with these hepatic residues. Linking defined oral doses of the SGAR brodifacoum with resulting hepatic brodifacoum concentrations and occurring effects in rainbow trout was the main objective of the present study. Such experimentally determined links are crucial for regulators in assessing the relevance of AR residues detected in wild fish, and in their decisions to implement risk mitigation measures to minimize AR exposure for fish. In order to account for different exposure situations, we conducted two separate experiments, covering an acute and a chronic/sub-chronic primary poisoning event. In a first experiment, trout were fed a single dose brodifacoum-spiked feed and observed for 15 d. In a second experiment, trout were exposed every 7 or 8 days to brodifacoum up to 60 d and samples were taken every 15th d. Given that vitamin K acts as an antidote to AR intoxication, its oversupplementation in commercial feed can limit the comparability to wild organisms (Rattner and Harvey, 2021). Hence, we used customized menadione-free fish feed in our study that contained naturally sufficient vitamin K_1 and K_2 to enable a physiological blood coagulation. Commonly applied tests to investigate the clotting ability of blood, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT; Bates and Weitz, 2005; Tavares-Dias and Oliveira, 2009), were applied to fish and endpoints related to anemia were assessed.

2. Material and methods

2.1. Test organism

Rainbow trout (*O. mykiss*) were cultivated at the Bavarian Environment Agency under disease-controlled conditions. In the 15 d-experiment, ca. 1-year old trout (198 ± 9 g, 26 ± 1 cm; Table A.1) were used, in the 60 d-experiment ca. 1.5-years old trout (326 ± 19 g, 30 ± 1 cm; Table A.1). A few weeks prior to the start of experiments, blood samples were collected from the vena cava caudalis under anesthesia (100 mg/L tricaine methanesulfonate for 5 min; Tricaine Pharmaq®, Pharmaq, Bergen, Norway). For sex determination, heparinized blood was centrifuged (1320 rcf, 10 min, 4 °C; plasma stored at −80 °C) and vitellogenin (Vtg) was analyzed in the plasma using a rainbow trout Vtg ELISA Kit (Prod. No. V01004402, Biosense Laboratories AS, Bergen, Norway; dilution 1:20). Additionally, a passive integrated transponder (PIT; AL-VET ID Minitransponder, Dechra, Germany) was injected into the muscle to mark individuals. The experiments were approved by the regional government of upper Bavaria (authorization number ROB-55.2–2532.Vet_02–20–192, 10. March 2021) and performed according to German legislation.

2.2. Test substance and preparation of spiked feed

Preceding chemical analyses revealed high levels of artificial vitamin K_3 (menadione) in commercially available fish feed. To avoid oversupply of fish with vitamin K during experiments, extruded feed without artificially added menadione was custom-made for this study by Simplyfish AS (Stavanger, Norway). The custom-made feed (3 mm) was designed to meet the nutritional requirements of growing rainbow trout by use of high-quality raw materials and contained naturally 3.15 μg/100 g vitamin K_1 and 3 μg/100 g vitamin K_2 (MK-4 and MK-7; Tables A.2 and A.3). Fish were fed the menadione-free feed a minimum of 60 d prior to the experiments. The hepatic vitamin K_1 level in the liver decreased from 130 ng/g in trout fed with commercial fish feed to 59 ng/g in trout fed menadione-free feed. Pretests indicated no adverse effects of the menadione-free feed on blood coagulation times.

To prepare the spiked feed, frozen (–80 °C) menadione-free feed was milled (Retsch ZM 200 with 500 μm sieve, Haan, Germany) and 40 g (15 d-experiment) or 300 g (60 d-experiment) feed were mixed with 10 % gluten as binding agent. Subsequently, the test substance was added. Homogenized, larval, red chironomid midges were filtered and the liquid thoroughly blended with the dough to increase attractiveness of the feed. The dough was coated with peanut oil during pelletization (in-house production). Afterwards, feed pellets (6 mm) were dried for 24 h at 37 °C. Control-feed without addition of test substance was prepared in the same way using separate equipment. In addition to strict cleaning

protocols, mostly disposable products were used to prevent contamination/carry-over of test substance during feed preparation.

Analytical grade brodifacoum (CAS: 56073–10–0; purity: 98.9 %) was purchased from LGC Standards GmbH (Wesel, Germany). For the 15 d-experiment, a stock solution (6.0928 mg brodifacoum dissolved in 100 mL acetone (purity ≥ 99.8 %; VWR Chemicals, Darmstadt, Germany)) was used to prepare feed concentrations up to 5.714 μg/g feed, whereas solid brodifacoum was added directly to the dough, when preparing higher concentrations. For the 60 d-experiment, a stock solution of 22.7 mg brodifacoum dissolved in 100 mL acetone was used. Chemical analysis ensured that acetone was completely evaporated from self-prepared feed pellets before they were fed to trout (Text A.1). Moreover, nominal brodifacoum concentrations in spiked and control-feed were verified by analytical measurements (Text A.2). As the deviation of nominal and measured brodifacoum concentration was less than 20 % in all of the prepared feed concentrations (Table A.4), nominal doses were used to report results in accordance with OECD recommendations (OECD, 2019a).

2.3. Experimental setup and sampling

Experiments were performed in a flow-through system supplied with a blend of reverse-osmosis permeate and aerated spring water (conductivity: 200 μs/cm, hardness: 6.3 °dH). The light/dark cycle was 12/12 h with 30 min twilight in the mornings and evenings. Tanks were regularly cleaned by removing feces and remaining feed to maintain water quality. Trout were acclimatized to water conditions for 48 h before they were randomly distributed to tanks, in which they were accustomed for at least 9 d. The sex ratio was comparable in all tanks and treatment groups. Tanks were randomly assigned to treatment groups in both experiments. One day before each experiment started, fish were anesthetized (anesthesia protocol provided in Text A.3), weighed, and measured, allowing for accurate calculation of brodifacoum doses to be administered (calculated according to Equation A.1). During experiments, the time until administered spiked-feed pellets were consumed by fish was measured for each tank. In addition, each regular feed uptake was assessed semi-quantitatively in 4 levels ascending from score 1–4 'no', 'severely reduced', 'reduced', 'good' for each fish (15 d-experiment) or as mean value for all fish per tank (60 d-experiment). Additionally, in the 60 d-experiment anorectic fish were separately documented. In accordance with OECD Guideline 203 (OECD, 2019b), the occurrence of clinical symptoms such as abnormal ventilation, abnormal swimming behavior (e. g., apathy), loss of equilibrium, hemorrhages, and other visible abnormalities was scored three times a day in fish from the 15 d-experiment and at least once a day in fish from the 60 d-experiment using a 3-level scoring system, ascending from score 0 'no symptom' to score 3 'high-grade'. At termination of exposure, sampling involved the collection of blood from the caudal vein (vena cava caudalis) of anesthetized fish (for details refer to Text A.3). After complete withdrawal of blood, death was ensured by cutting the spine. The body condition (external and internal) of each fish was evaluated and tissue samples were taken. Collected blood samples were immediately processed. Serum, liver, and muscle (homogenized filet) tissue samples for chemical analysis of brodifacoum residues were stored at −80 °C until processing.

Trout deceased during experiments were removed from tanks and sampled if conditions allowed. Moribund fish (i.e., loss of ability to swim coordinately, loss of equilibrium, or high-grade apathy) that met previously defined termination criteria were euthanized to reduce the period of suffering, and sampled accordingly. Dead fish and euthanized moribund fish were summarized in the parameter mortality (Table A.5).

2.3.1. 15 d-experiment (single dose administered)

Trout were housed individually in 80 tanks. Each tank (27 L water volume) was equipped with an in- and outflow (flow rate: 20 L/h), and aeration. Physico-chemical water parameters at the inflow (temperature: 11.5 ± 0.1 °C, pH: 7.3 ± 0.0, conductivity: 197.7 ± 1.3 μS/cm) were monitored continuously (IQ Sensor Net: System 2020 3 G; Xylem, Weilheim, Germany). Oxygen (O_2: average concentration: 8.6 ± 0.4 mg/L), nitrite (NO_2^-: < 49 μg/L), and ammonia (NH_3: max. 1.0 μg/L, mean 0.3 ± 0.2 μg/L; calculated based on measured total ammonium concentration according to Hobiger 1996) levels were checked once a week in each tank. Each of the eight treatment groups (referred to as groups 1_{15}–8_{15} in the following) comprised of ten individually housed trout. Fish were administered a single dose of brodifacoum-spiked feed (groups 1_{15}–8_{15}: 0; 0.64; 3.2; 16; 80; 400; 2000; 10,000 μg/kg bw) at day 0 of the 15 d-experiment. The dose was mixed in the feed equivalent to 1.4 % bw and administered in two portions. To exclude leaching of test substance from spiked feed, pellets were fed one by one. Starting day 1, control-feed (1 % of bw, divided in two portions) was fed daily over the runtime of the experiment. At sampling on day 15, coagulation times, hematocrit, and albumin level, as well as brodifacoum residues in serum and liver were assessed in every fish, and exemplarily in 2 muscle tissue samples per treatment group.

2.3.2. 60 d-experiment (multiple doses administered)

Trout were kept in groups ($n = 20 + 2$ additional fish per tank) in 16 aerated tanks filled with 600 L water (flow rate: 100 L/h). Water quality parameters were continuously monitored in the inflow (temperature: 11.7 ± 0.4 °C, pH: 7.4 ± 0.0, conductivity: 198.0 ± 1.2 μS/cm) and outflow (oxygen saturation: 92 ± 3 %, min. 80 %). Ammonia and nitrite levels were determined twice a week (calculated NH_3: max. 3.2 μg/L, mean 1.5 ± 0.6 μg/L; NO_2^-: < 49 μg/L). One control group and seven treatment groups were assessed in duplicate. Starting at day 0, trout were administered multiple doses of brodifacoum-spiked feed (1 % of bw, divided in two portions) every 7 or 8 days, i.e., fish were administered a total of 8 brodifacoum doses over 60 d (Table 1). In-between administered brodifacoum doses, fish were fed daily with menadione-free, unprocessed feed (1 % of bw, divided in two portions). Self-prepared control-feed was solely fed the day before administration of brodifacoum doses to prevent feed refusal by fish (i.e., due to its change in pellet size and taste). Every 15 d, all fish were measured and weighed under anesthesia and five randomly selected trout per tank were removed for sampling as detailed in Text A.3. Based on the weight measurements, the daily feed quantity was recalculated prior to the next administration of brodifacoum-spiked feed to consider growth of trout (Equation A.1).

2.4. Blood and tissue analyses

2.4.1. Blood coagulation times

Citrated blood (S-Monovette® Citrat 9NC 0.106 mol/L 3.2 %, 1.4 mL, Sarstedt, Nümbrecht, Germany) was centrifuged (1160 rcf, 10 min, 4 °C) and the plasma stored on ice prior to analyses. PT, aPTT and TT were measured with a BFT II Analyzer (Siemens Healthcare, Erlangen, Germany). For PT and aPTT, manufacturers instructions had to be modified as follows to analyze fish plasma. 360 μL Thromborel®S was mixed with 90 μL 0.05 mol/L $CaCl_2$ directly before the PT assay. 50 μL plasma were incubated for 60 s (37 °C). Following, 200 μL of the $CaCl_2$-Thromborel®S mixture was added and the time it took the sample to clot was measured. To analyze aPTT, 50 μL plasma were mixed with 50 μL pathromtin and incubated for 120 s (37 °C). 100 μL 0.05 mol/L $CaCl_2$ was added and aPTT determined. TT was assessed according to manufacturers instructions. All reagents were purchased from Siemens Healthcare. For each assay, samples were analyzed in duplicate and the measurement interrupted at 1200 s. Only values classified as valid were used for subsequent data analysis (maximal relative deviation of the individual values PT: 11.5 %, aPTT: 10.5 %, TT: 10 %; RiliBÄK, 2022).

2.4.2. Hematocrit and albumin level

Two hematocrit sodium heparinized capillaries (Hirschmann

Table 1
Cumulative nominal brodifacoum doses in μg/kg body weight of the treatment groups and investigated endpoints at the four samplings during the 60 d-experiment. NA = not applicable (no remaining fish in the treatment group).

	Period 1 (day 0 – 15)		Sampling 1	Period 2 (day 16 – 30)		Sampling 2	Period 3 (day 31 – 45)		Sampling 3	Period 4 (day 46 – 60)		Sampling 4
	1. dose day 0	2. dose day 8	Day 15	3. dose day 16	4. dose day 23	Day 30	5. dose day 31	6. dose day 38	Day 45	7. dose day 46	8. dose day 53	Day 60
Group 1_{60}	0.00	0.00	Coagulation times[a], hematocrit, chemical analysis	0.00	0.00	Coagulation times, hematocrit, albumin level, chemical analysis	0.00	0.00	Coagulation times[b], hematocrit, chemical analysis	0.00	0.00	Coagulation times, hematocrit, albumin level, chemical analysis
Group 2_{60}	0.78	1.56		2.34	3.13		3.91	4.69		5.47	6.25	
Group 3_{60}	1.56	3.13		4.69	6.25		7.81	9.38		10.94	12.50	
Group 4_{60}	3.13	6.25		9.38	12.50		15.63	18.75		21.88	25.00	
Group 5_{60}	6.25	12.50		18.75	25.00		31.25	37.50		43.75	50.00	
Group 6_{60}	12.50	25.00		37.50	50.00		62.50	75.00		87.50	100.00	
Group 7_{60}	25.00	50.00		75.00	100.00		125.00	150.00		NA	NA	
Group 8_{60}	50.00	100.00		150.00	200.00		NA	NA		NA	NA	

[a] Only analyzed in 1 replica (5 fish) per treatment group.
[b] Only analyzed in 1 replica (5 fish) per treatment group except group 1_{60} and group 6_{60} (all fish).

Laborgeräte, Eberstadt, Germany) per sample were filled with lithium-heparin (lithium heparin LH, 1.3 mL, Sarstedt, Nümbrecht, Germany) whole blood, one end sealed, and centrifuged (11,696 rcf, 10 min, room temperature). The hematocrit values were derived with the Hawksley microhematocrit reader (Hawksley & Sons, Lancing, UK). To analyze the albumin level, heparinized blood was centrifuged (1320 rcf, 10 min, 4 °C). Albumin level was determined in the plasma with a fully automatic clinical chemical analysis system (diagnosis II, element RC3X, scil animal care company, Viernheim, Germany).

2.4.3. Residues of brodifacoum

Chemical analysis of brodifacoum in liver and muscle tissue samples was done by liquid chromatography-tandem mass spectrometry (4500 QTrap, Sciex, Darmstadt, Germany) after ultra-sound assisted solvent extraction and dispersive solid phase extraction clean-up as detailed in Regnery et al. (2019b). The clean-up and enrichment steps were omitted during extraction of tissue samples from treatment groups that had received high brodifacoum doses (i.e., groups 5_{15}–8_{15} in the 15 d-experiment, groups with a cumulative dose ≥ 6.25 μg/kg bw in the 60 d-experiment). Serum samples were extracted following the method by Dong et al. (2015). In all samples, brodifacoum was quantified using an isotope-labeled internal standard (brodifacoum-d4, TRC, North York, Ontario, Canada). Extracts of samples with residual brodifacoum concentrations that exceeded the linear 9-point calibration standard range of 0.05–40 ng/mL (correlation coefficient $r > 0.99$) were appropriately diluted prior analysis. Previously established and validated method quantification limits of brodifacoum were 0.9 ng/g in liver tissue, 0.3 ng/g in muscle tissue, and 0.8 ng/mL in serum (Dong et al., 2015; Regnery et al., 2019b). All reported brodifacoum concentrations in liver and muscle tissue are based on wet weight.

2.5. Credibility of data and statistic

The reporting criteria for ecotoxicity studies (CRED) proposed by Moermond et al. (2016) are provided in the Appendix A, supplementary file 2. Six fish were excluded due to causes unrelated with the experiment itself (Table A.6). Statistical analyses were performed with R (version 4.3.1, R Core Team, 2023). The α-level was set to 0.05. If necessary, data were transformed to gain normal distribution and homogeneity of variances. If possible, (mixed) linear models (package "lme4"; Bates et al., 2015) were performed including "tank" as a random factor in the 60 d-experiment. Treatment groups with ≤ 3 fish were excluded from statistical analyses. Furthermore, no statistical analysis of brodifacoum residues in serum was performed for a total dose of 25 μg/kg bw brodifacoum, because group 4_{60} comprised values below the quantification limit. Sex was included as a fixed factor in the analyses of the coagulation times in the 15 d-experiment. For pairwise comparisons with the control group (all analyses except comparisons of the same total dose of different groups at different samplings), the Dunnett`s test was used. Treatment groups with the same cumulative dose at different samplings were compared with Tukey contrasts. If the data distribution did not allow to perform a mixed linear model, a Kruskal-Wallis rank sum test followed by a Wilcoxon rank sum exact test, adjusted with Benjamini-Hochberg for pairwise comparisons, was used. However, it was not possible to consider the pseudo-replication of the 60 d-experiment in the non-parametric test. The coagulation data are censored at 1200 s. In the 15 d-experiment, an analysis of the coagulated versus not coagulated PT was performed with Firth regression (package "logistf"; Heinze et al., 2023) with a Benjamini-Hochberg post-hoc correction. Subsequently, the coagulated data were analyzed with a linear model. The endpoint mortality was not analyzed with a statistical model because of the differing total brodifacoum doses. For an overview, hepatic residue concentrations of both experiments were plotted against the normalized cumulative dose. A polynomial regression was performed as a simple model to compare exposure and hepatic residue concentrations between the two experiments. No data were extrapolated based on the simplified model. Additional information regarding statistical analyses is provided in Tables A.7 and A.8.

3. Results

3.1. Uptake of brodifacoum-spiked feed

All trout consumed their administered complete dose of brodifacoum-spiked feed. No significant differences in the measured feeding duration of the complete daily doses of brodifacoum-spiked feed occurred in both experiments (15 d-experiment: $F = 1.655$, $d.f. = 7|72$, $p = 0.133$; 60 d-experiment: $F = 0.934$, $d.f. = 7|107$, $p = 0.484$). Feeding of one pellet in the 15 d-experiment and one portion of brodifacoum-spiked feed in the 60 d-experiment took on average 2.5 s and 102 s,

respectively. As the time until almost complete uptake of brodifacoum-spiked feed in group 7_{60} amounted to several hours on day 38 (6th feeding) these data were excluded from statistical analysis.

3.2. Observed effects during the experiments and mortality

In the first 15 d of both experiments, none of the fish died and no clinical symptoms were evident with exception of a severe hemorrhage from the gills of one trout (group 7_{15}, 2000 µg/kg bw) after 14 d. The hemorrhage caused no dyspnea. Furthermore, no mortality occurred in group 1_{60} to group 6_{60} during the entire exposure period. However, in group 8_{60} the first fish died after 17 d and in group 7_{60} after 28 d at a cumulative dose of 150 µg/kg bw and 100 µg/kg bw, respectively. 5 surviving fish of group 8_{60} were sampled after 30 d and 2 surviving fish of group 7_{60} after 45 d. Mortality of group 7_{60} and group 8_{60} is summarized in Table 2.

Most deceasing fish showed increasing signs of discoloration, apathy, anorexia, and tumbling movement, often lasting over several days. Apathy was solely observed in group 7_{60} (days 32–45, up to 100 % with score 3) and group 8_{60} (days 18–30, up to 100 % with score 2 and 50 % with score 3). Likewise, reduced feed intake occurred in group 7_{60} (onset on day 32, mean score 3.2 ± 0.52, period 3, day 32–45) and group 8_{60} (onset on day 24, mean score 3.0 ± 1.12, period 2, day 17–30) with up to 100 % of anorectic fish completely refusing feed uptake (all other groups$_{15/60}$ with mean score 4.0 ± 0.0–0.2). Anorexia was solely in group 8_{60} reflected by a significantly reduced weight gain of 42 ± 21 g between 15 d and 30 d of the 60 d-experiment compared to a mean increase of 75 ± 12 g in the control group ($F = 3.677$, $d.f. = 7|7.8386$, Dunnett: $p < 0.001$; Tables A.9 and A.10). Additionally, exophthalmos or hemorrhages were observed in some fish. However, other fish showed no clinical symptoms and died within a few hours. Moribund fish had pale gills, lost control of equilibrium, and were not able to swim coordinately.

3.3. Blood coagulation times

In the 15 d-experiment, the PT of fish exposed to 80 µg/kg bw brodifacoum (group 5_{15}) was significantly prolonged and more than twice as long as in the control group (Fig. 1 and Table A.11; $F = 5.645$, $d.f. = 4|44$, $p < 0.001$). Higher doses of brodifacoum (groups 6_{15}–8_{15}) in the 15 d-experiment led to a mean PT > 1000 s. Likewise, doses of ≥ 400 µg/kg bw brodifacoum significantly prolonged aPTT by 41–54 % (Table A.11; $F = 16.034$, $d.f. = 7|71$, $p < 0.001$). In contrast, TT was not affected by exposure to brodifacoum (Table A.11; $F = 0.985$, $d.f. = 7|71$, $p = 0.449$). Sex had no influence on blood clotting of fish with a measurable coagulation time (PT: $F = 0.004$, $p = 0.948$; aPTT: $F = 0.622$, $p = 0.433$; TT: $F = 0.497$, $p = 0.483$). However, slightly more female fish had a PT > 1200 s (Fig. A.1; $X^2 = 54.012$, $p = 0.047$).

Exact values for PT, aPTT, and TT of the 60 d-experiment are provided in Table A.12. After 15 d of the 60 d-experiment, 80 % of the analyzed fish in group 8_{60} (100 µg/kg bw) had a PT > 1000 s (Fig. 2.1). The corresponding values for aPTT and TT were not affected. In the second sampling (Fig. 2.2) , no PT was measurable in all surviving fish of group 8_{60} (200 µg/kg bw) and 9 of 10 fish of group 7_{60} (100 µg/kg bw).

Table 2
Cumulative mortality [%] of group 7_{60} and group 8_{60}during the 60 d-experiment. NA = not applicable (no fish remained in the treatment group).

	Mortality 16–22 d (dose)	Mortality 23–30 d (dose)	Mortality 31–37 d (dose)	Mortality 38–45 d (dose)
Group 7_{60}	0 % (75 µg/kg bw)	13 % (100 µg/kg bw)	77 % (125 µg/kg bw)	91 % (150 µg/kg bw)
Group 8_{60}	21 % (150 µg/kg bw)	85 % (200 µg/kg bw)	NA	NA

Furthermore, the mean aPTT was significantly increased by 36 % in group 7_{60} and considerably prolonged or not measurable in group 8_{60} ($X^2 = 35.064$, $d.f. = 7$, $p < 0.001$). On the contrary, TT values indicated that fibrin was formed significantly faster in group 8_{60} compared to controls ($F = 3.431$, $d.f. = 7|8.4482$, $p = 0.049$). After 45 d (Fig 2.3), the PT was not measurable in both surviving fish of group 7_{60} (150 µg/kg bw), and their aPTT was increased by 72 %. While one fish of group 6_{60} (75 µg/kg bw) had a PT above 1200 s, PT was significantly prolonged by on average 40 % in all other fish of group 6_{60} ($F = 10.173$, $d.f. = 1|17$, $p = 0.005$). At the end of the experiment (Fig. 2.4), out of 14 fish of group 6_{60} (100 µg/kg bw) the PT of 4 was not measurable, the PT of 3 trout was more or less prolonged, while the PT of 7 fish was within the range of the control group ($F = 6.484$, $d.f. = 5|5.7436$, $p = 0.023$). The aPTT of group 6_{60} was significantly prolonged by 20 %, whereas no difference occurred in the TT of all treatment groups (aPTT: $X^2 = 14.608$, $d.f. = 5$, $p = 0.012$).

3.4. Hematocrit and albumin level

In the 15 d-experiment, the mean hematocrit value of all fish was 35 ± 4 %. Except for the fish with gill bleeding (group 7_{15}, hematocrit of 12 %), both the hematocrit and albumin level (total average 2.7 ± 0.2 g/dL) were not affected by the single dose brodifacoum after 15 d (Table A.13). However, at the second sampling of the 60 d-experiment, hematocrit values had decreased slightly to 33 ± 12 % in group 7_{60} and significantly to only 4 ± 4 % in group 8_{60} (Table A.14; $F = 13.655$, $d.f. = 7|8.318$, $p = 0.001$) compared to the control group (hematocrit of 41 ± 4 %). Furthermore, the albumin level of group 8_{60} was 1.4 ± 0.4 g/dL and, thereby, significantly lower than the control group (2.7 ± 0.1 g/dL) after 30 d of exposure to brodifacoum (Table A.14; $F = 10.251$, $d.f. = 7/67.493$, $p < 0.001$). In moribund fish the average hematocrit was 2.7 ± 1.0 % (max. 4.5 %), and the mean albumin level was 1.0 ± 0.3 g/dL. In groups 2_{60}–6_{60}, neither hematocrit nor albumin levels were affected by exposure to brodifacoum during the entire experiment.

3.5. Chemical analyses

No residues of brodifacoum were detected above the respective quantification limits in serum, liver, and muscle tissue samples of fish from control groups in either of the two experiments (Table 3). In all other treatment groups of both experiments, brodifacoum was detected in every analyzed liver tissue sample. Hepatic concentrations followed a second-order polynomial regression against the normalized cumulative dose ($\log_{10}(x + 1)$ with a coefficient of determination of $R^2 = 0.8$ (Fig. A.2)). In livers of euthanized and deceased fish, brodifacoum concentrations ranged between 71 and 826 ng/g (wet weight) with an average concentration of 370 ± 178 ng/g. Exposure of fish of different treatment groups to the same cumulative dose in the 60 d-experiment led to on average similar mean hepatic brodifacoum concentrations (Fig. 3 and Table A.15; 6.25 µg/kg bw: $F = 0.274$, $d.f. = 2|30$, $p = 0.762$; 12.5 µg/kg bw: $F = 0.2406$, $d.f. = 2|2.617$, $p = 0.802$; 25 µg/kg bw: $F = 6.233$, $d.f. = 2|2.528$, $p = 0.105$). However, higher cumulative brodifacoum doses resulted in more variable residues in the liver with significant differences at a cumulative dose of 100 µg/kg bw brodifacoum (50 µg/kg bw: $F = 8.200$, $d.f. = 2|2.857$, $p = 0.066$; 100 µg/kg bw: $X^2 = 8.300$, $d.f. = 2$, $p = 0.016$). In groups 1_{15}–4_{15} and groups 1_{60}–4_{60} no brodifacoum was detected in the serum of trout (Table 3). In the 60 d-experiment, the highest observed brodifacoum concentration in blood was 32 ng/mL, whereas up to 1860 ng/mL brodifacoum were detected in trout of group 8_{15} in the 15 d-experiment. In the 60 d-experiment, the brodifacoum concentration in serum of fish from different treatment groups exposed to the same cumulative dose decreased with increasing number of lower single doses (Fig. 4 and Table A.15; 50 µg/kg bw: $F = 3.886$, $d.f. = 2|30$, $p = 0.032$; 100 µg/kg bw: $X^2 = 20.67$, $d.f. = 2$, $p < 0.001$). In both experiments, low residues of brodifacoum were found in exemplarily analyzed muscle tissue at a cumulative dose of

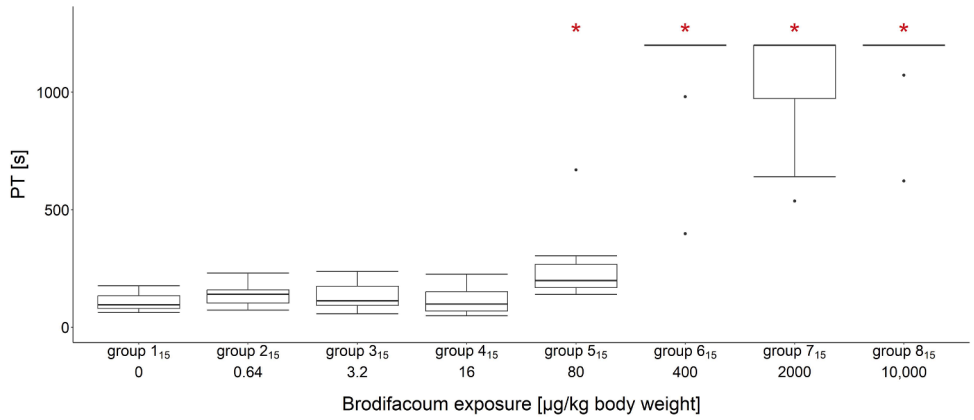

Fig. 1. Prothrombin time (PT) in 15 d-experiment (all groups $n = 10$). The boxplots depict the 25th and 75th percentile, the median, as well as the minimum and maximum values (whiskers). Outliers are displayed as dots. Asterisks highlight significant differences compared to the control group ($p < 0.05$, exact values see Table A.11).

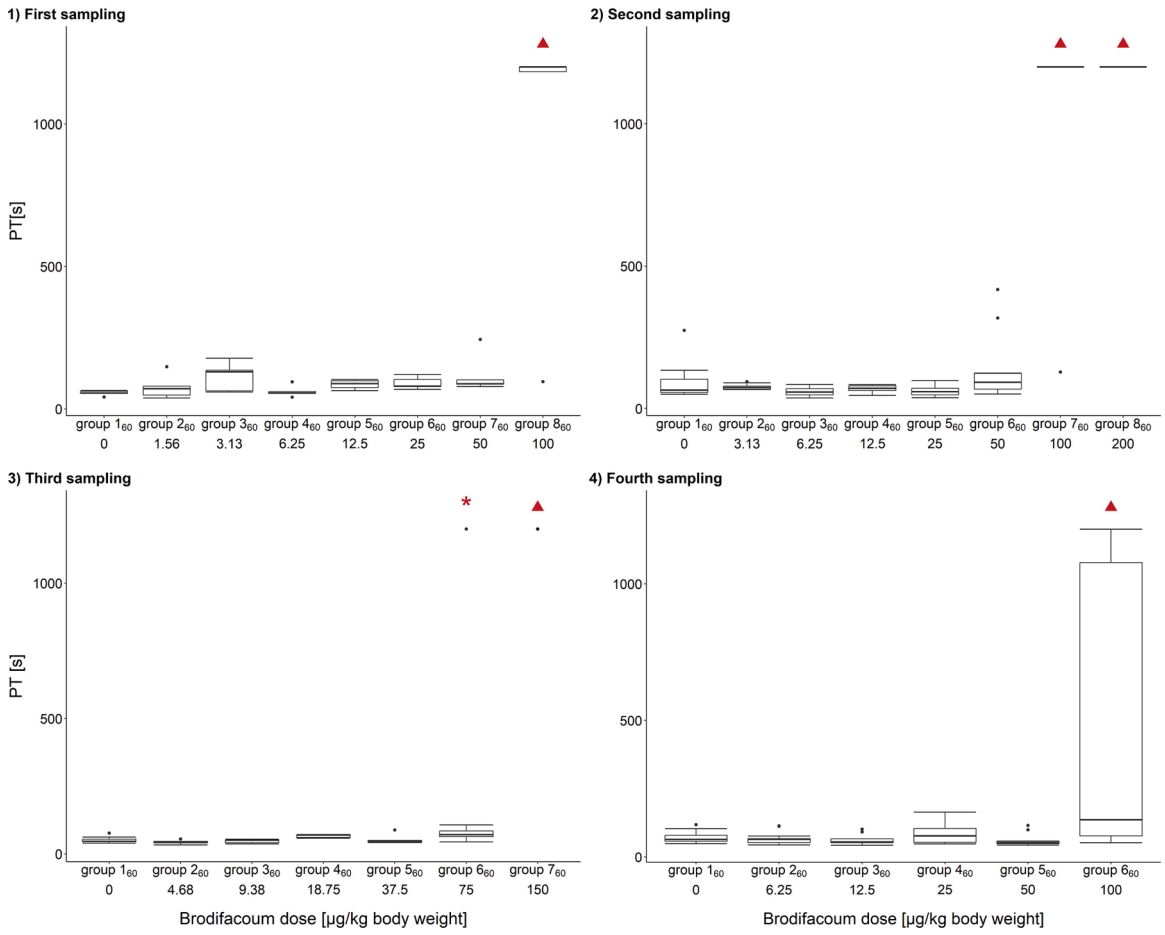

Fig. 2. Prothrombin time (PT) at each of the four samplings in the 60 d-experiment. 1): First sampling (15 d; all groups $n = 5$). 2): Second sampling (30 d; group 1_{60}–7_{60} $n = 10$, group 8_{60} $n = 5$). 3): Third sampling (45 d; group 1_{60} and group 6_{60} $n = 10$, group 2_{60}–5_{60} n = 5, group 7_{60} $n = 2$). 4): Fourth sampling (60 d; group 1_{60}–3_{60} and group 5_{60} $n = 13$, group 4_{60} and group 6_{60} $n = 14$). The boxplots depict the 25th and 75th percentile, the median, as well as the minimum and maximum values (whiskers). Outliers are displayed as dots. An asterisk highlights significant differences compared to the control group ($p < 0.05$; exact values see Table A.12). Triangles highlight mean PT of more than fivefold increase compared to the control group.

$\geq$ 3.13 µg/kg bw (Table 3). The highest observed concentration in a euthanized trout (150 µg/kg bw brodifacoum) was 8.5 ng/g muscle.

4. Discussion

Conducting aquatic toxicity experiments with SGARs is demanding because of their specific mode of action and challenging substance properties. The strong adsorption and low water solubility of brodifacoum suggest oral uptake as the primary route of wild fish exposure. Successful oral administration of the test substance is therefore a prerequisite to testing effects of brodifacoum under environmentally relevant conditions. In our study, rainbow trout consumed spiked pellets

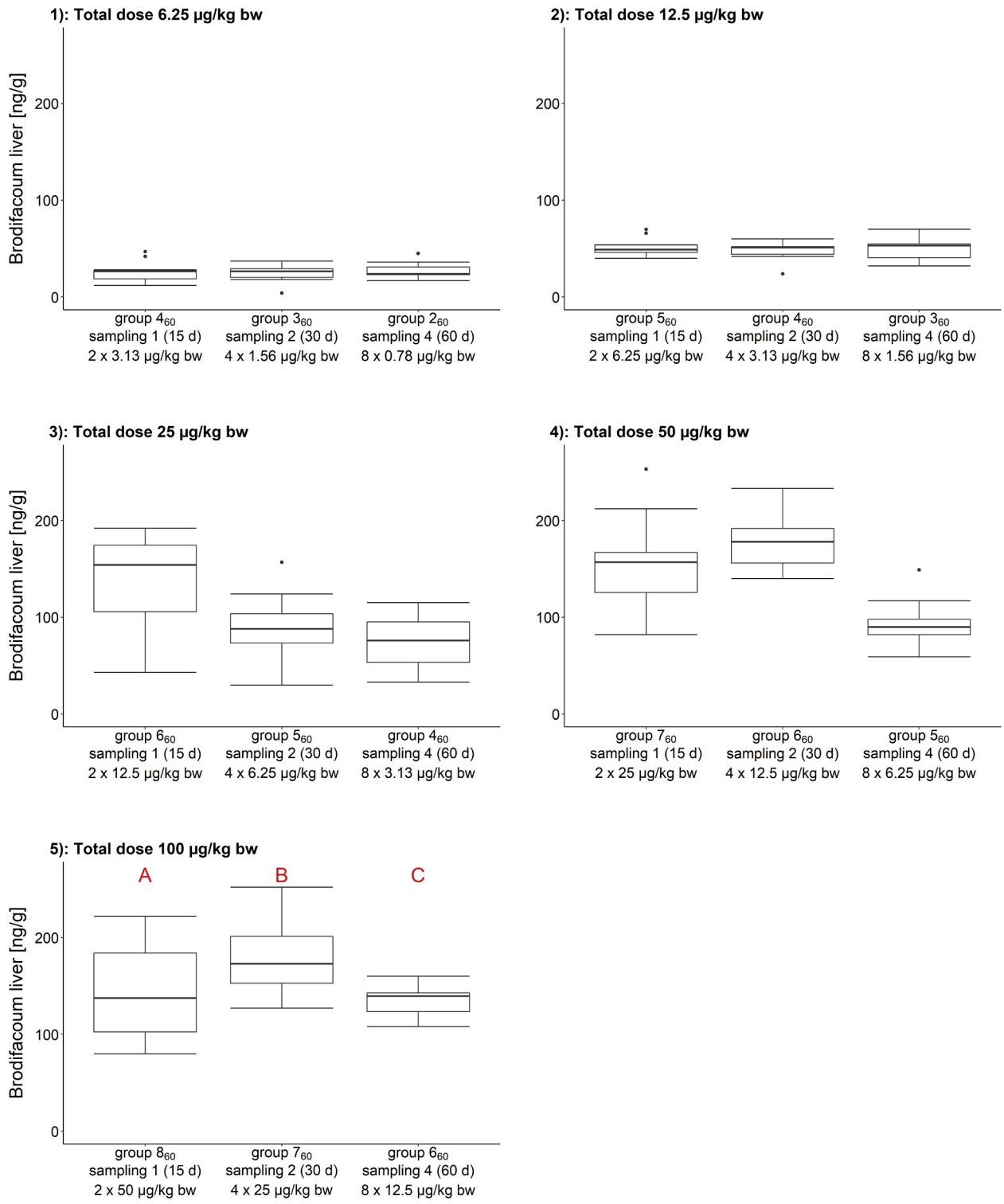

Fig. 3. Comparison of residues in the liver of different treatment groups with the same cumulative brodifacoum dose at different samplings (sampling 1: 2 doses of brodifacoum, sampling 2: 4 doses of brodifacoum, sampling 4: 8 doses of brodifacoum). The boxplots depict the 25th and 75th percentile, the median, as well as the minimum and maximum values (whiskers). Outliers are displayed as dots. 1): total dose of 6.25 µg/kg bw brodifacoum. 2): total dose of 12.5 µg/kg bw brodifacoum. 3): total dose of 25 µg/kg bw brodifacoum. 4): total dose of 50 µg/kg bw brodifacoum. 5): total dose of 100 µg/kg bw brodifacoum. Different capital letters depict significant differences between the groups. Further statistical information is provided in Tables 3 and A.8.

within seconds and no effect on palatability was observed. The delay of 14–17 d until onset of first overt symptoms of a coagulation disorder in trout (cold water fish) was noticeably longer compared to usually 2–10 d previously reported in terrestrial species (Rached et al., 2020; Rattner et al., 2014b; Rattner and Mastrota, 2018). This clearly emphasizes the importance of the testing duration (length of observation period) when exposing aquatic organisms to slow acting SGARs. It also highlights the benefits of preliminary testing prior to experiments to optimize the study design for such challenging substances, preventing potential mis-interpretations in terms of sensitivity (e.g., due to overdosing) and thus

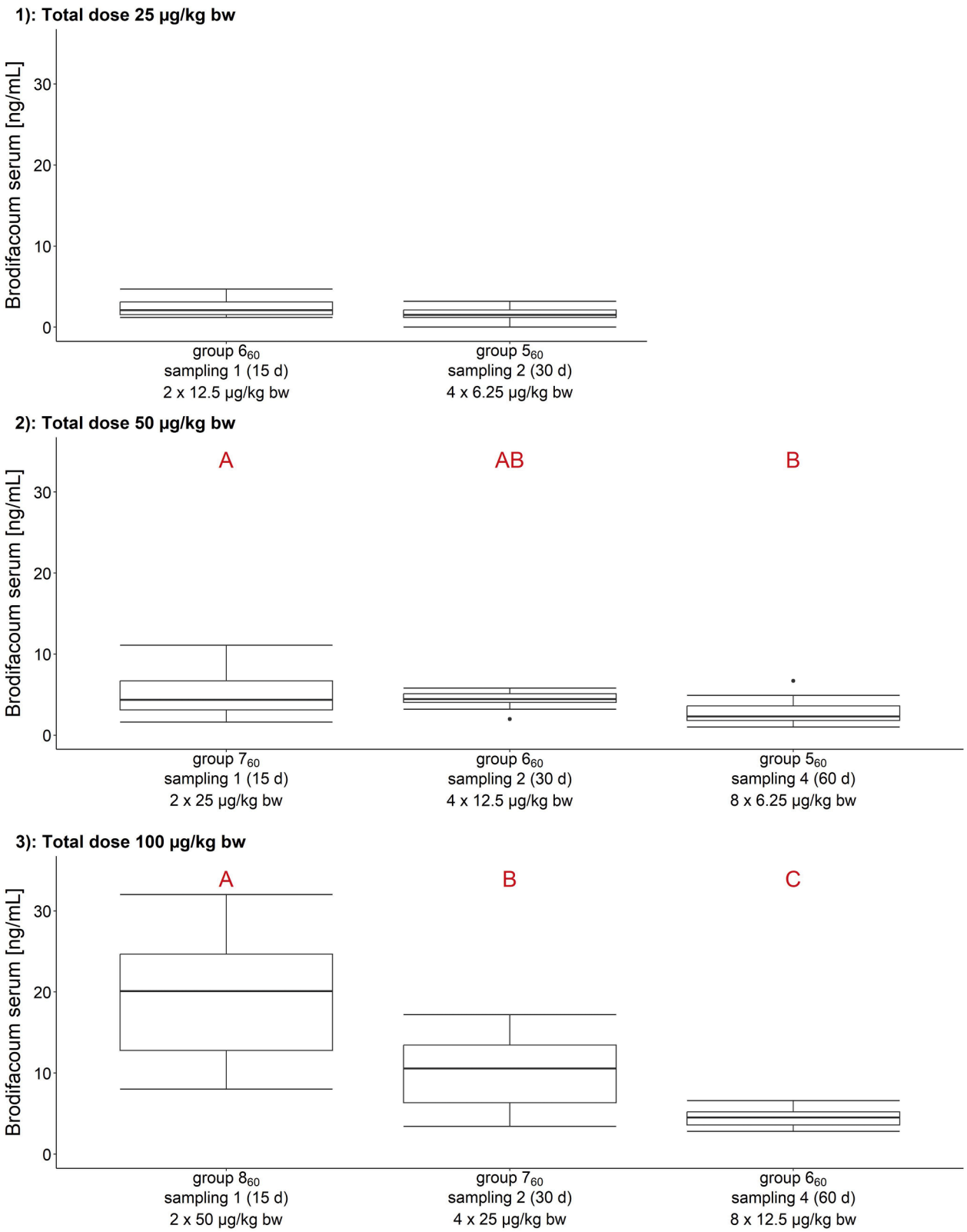

Fig. 4. Comparison of residues in the blood serum of different treatment groups with the same cumulative brodifacoum dose at different samplings (sampling 1: two doses of brodifacoum, sampling 2: four doses of brodifacoum, sampling 4: eight doses of brodifacoum). The boxplots depict the 25th and 75th percentile, the median, as well as the minimum and maximum values (whiskers). Outliers are displayed as dots. 1): total dose of 25 µg/kg bw brodifacoum (sampling 4 of group 4_{60} is not depicted as all values were < LOQ). 2): total dose of 50 µg/kg bw brodifacoum. 3): total dose of 100 µg/kg bw brodifacoum. Different capital letters depict significant differences between the groups. Further statistical information is provided in Tables 3 and A.8.

Table 3
Overview of measured brodifacoum residues in liver and serum (mean ± standard deviation, individual values or if some values < LOQ (limit of quantification) range of values) and muscle (individual values) of fish; LOQ liver = 0.9 ng/g; LOQ serum = 0.8 ng/mL; LOQ muscle = 0.3 ng/g; NA = not applicable (no remaining fish in the treatment group); Cum. brod. dose: Cumulative brodifacoum dose.

	Sampling	Cum. brod. dose [µg/kg bw]	*n* liver | serum | muscle	Brodifacoum concentration		
				Liver [ng/g]	Serum [ng/mL]	Muscle [ng/g]
Group 1_{15}	15 d	0	10 | 10 | 2	< LOQ	< LOQ	< LOQ; < LOQ
Group 2_{15}	15 d	0.64	10 | 10 | 2	6.2 ± 1.1	< LOQ	< LOQ; < LOQ
Group 3_{15}	15 d	3.2	10 | 10 | 2	16.4 ± 4.4	< LOQ	< LOQ; < LOQ
Group 4_{15}	15 d	16	10 | 10 | 2	130.8 ± 32.7	< LOQ	< LOQ; 1.1
Group 5_{15}	15 d	80	10 | 10 | 2	279.6 ± 103.2	4.8 ± 2.1	2.1; 2.1
Group 6_{15}	15 d	400	10 | 10 | 2	359.3 ± 56.5	68.4 ± 20.1	3.8; 4.4
Group 7_{15}	15 d	2000	10 | 10 | 2	535.5 ± 235.0	334.6 ± 102.1	16; 17
Group 8_{15}	15 d	10,000	10 | 10 | 2	866.0 ± 377.2	1145.3 ± 404.0	29; 46
Group 1_{60}	15 d	0	11 | 11 | 2	< LOQ	< LOQ	< LOQ; < LOQ
	30 d	0	10 | 10 | 2	< LOQ	< LOQ	< LOQ; < LOQ
	45 d	0	10 | 10 | 2	< LOQ	< LOQ	< LOQ; < LOQ
	60 d	0	13 | 13 | 2	< LOQ	< LOQ	< LOQ; < LOQ
Group 2_{60}	15 d	1.56	10 | 10 | 2	10.3 ± 2.3	< LOQ	< LOQ; < LOQ
	30 d	3.13	10 | 10 | 2	18.1 ± 5.4	< LOQ	< LOQ; < LOQ
	45 d	4.68	10 | 10 | 2	17.5 ± 1.5	< LOQ	0.4; 0.6
	60 d	6.25	13 | 13 | 2	26.9 ± 8.0	< LOQ	0.5; 1.0
Group 3_{60}	15 d	3.13	10 | 10 | 2	15.7 ± 4.6	< LOQ	0.4; 0.7
	30 d	6.25	10 | 10 | 2	24.2 ± 9.1	< LOQ	0.5; 0.6
	45 d	9.38	10 | 10 | 2	41.8 ± 6.3	< LOQ	1.1; 1.8
	60 d	12.5	14 | 14 | 2	49.6 ± 11.9	< LOQ	0.9; 1.1
Group 4_{60}	15 d	6.25	10 | 10 | 2	26.6 ± 11.0	< LOQ	0.4; 0.7
	30 d	12.5	10 | 10 | 2	47.7 ± 10.1	< LOQ –1.4	0.6; 0.8
	45 d	18.75	10 | 10 | 2	58.0 ± 12.3	< LOQ	1.3; 1.5
	60 d	25	14 | 14 | 2	73.1 ± 25.5	< LOQ	1.4; 1.7
Group 5_{60}	15 d	12.5	10 | 10 | 2	51.7 ± 9.5	< LOQ –1.6	1.7; 1.9
	30 d	25	10 | 10 | 2	89.2 ± 36.0	< LOQ –3.2	0.7; 1.7
	45 d	37.5	10 | 10 | 2	97.8 ± 23.0	< LOQ –5.6	1.2; 1.3
	60 d	50	13 | 13 | 2	93.7 ± 22.7	2.8 ± 1.6	0.9; 1.3
Group 6_{60}	15 d	25	10 | 10 | 2	136.0 ± 49.0	2.5 ± 1.3	2.5; 2.7
	30 d	50	10 | 10 | 2	178.7 ± 28.9	4.3 ± 1.1	2.9; 4.4
	45 d	75	10 | 10 | 2	122.6 ± 27.1	3.1 ± 1.0	2.1; 3.0
	60 d	100	14 | 13 | 2	135.6 ± 15.3	4.5 ± 1.1	3.0; 3.4
Group 7_{60}	15 d	50	10 | 10 | 2	155.9 ± 50.1	5.1 ± 3.0	3.0; 3.8
	30 d	100	10 | 10 | 2	181.5 ± 39.2	9.9 ± 4.5	3.8; 4.2
	45 d	150	2 | 2 | 2	192.8; 401.2	5.0; 9.7	2.2; 2.8
	60 d	NA	NA	NA	NA	NA
Group 8_{60}	15 d	100	10 | 10 | 2	143.6 ± 50.6	19.4 ± 7.8	3.3; 4.0
	30 d	200	5 | 5 | 2	146.0 ± 59.6	18.1 ± 4.8	4.8; 5.0
	45 d	NA	NA	NA	NA	NA
	60 d	NA	NA	NA	NA	NA

underestimation of effects. Our results demonstrate that testing effects of SGARs on rainbow trout, and most likely also on other fish species, requires species-dependent test durations and cannot be assessed using current standard biotests. In addition, the fact that commercial fish diet is generally highly supplemented with vitamin K, most likely not reflecting the environmental vitamin K intake of fish, requires attention. So far, no test guideline accounts for the very specific mode of action of SGARs and considers vitamin K in commercial diet, which acts as an antidote against AR intoxication.

4.1. *Effects of brodifacoum exposure*

In the 60 d-experiment, doses of 100–200 µg/kg bw brodifacoum caused high mortality rates from day 17 onwards (Table 2). Although no mortality occurred in the 15 d-experiment even at much higher doses of 10,000 µg/kg bw brodifacoum, it is reasonable to conclude from the data of the 60 d-experiment that fish exposed to > 80 µg/kg bw brodifacoum would have died of coagulopathy during an extended observation period. The results of the experiments support the evidence that the mode of action of ARs in fish is comparable to that of mammals and birds (Fernández et al., 2014; Granadeiro et al., 2019; Jung and Kawatsu, 1995; Riegerix et al., 2020; Salte and Norberg, 1991). Brodifacoum-exposed fish showed dose-dependent typical symptoms of an intoxication with ARs such as prolonged clotting times, hemorrhages, discoloration, anemia, lethargy, and anorexia (Rattner et al., 2014b; Scott and Sloman, 2004; Valchev et al., 2008; Webster et al., 2015). Coagulation times were assessed with a method developed for human blood adapted for fish. In our study, the physiological magnitude and variability of PT in trout was higher than in other vertebrates (i.e., up to a twofold increase in mean PT in different control groups). Consequently, a 25 % increase of PT, as suggested in literature as an indication of AR intoxication (Rattner et al., 2014b), was not suitable in our experiments. However, the dose-dependent effects on the PT were clear and consistent in our study (Figs. 1 and 2). Clinical symptoms such as apathy, anorexia, hemorrhages and mortality caused by brodifacoum exposure occurred in test groups in which at least a fivefold increase of PT was observed compared to the corresponding control group. Therefore, significant differences of PT or a fivefold increase compared to controls, respectively, were interpreted as a pathological and thus biological relevant effect. In both experiments, 75 or 80 µg/kg bw brodifacoum caused a prolonged but still measurable PT. Higher doses of brodifacoum led in each case to a severely impaired hemostasis following a steep dose-response curve similar to those reported for other species (Rattner et al., 2014a; Rattner and Mastrota, 2018). As described for humans, the aPTT was also affected by exposure to brodifacoum, but less sensitive than the PT assay (Bates and Weitz, 2005). The unaffected TT excludes a deficiency of fibrinogen or a dysfibrinogenemia. The

physiological TT substantiates nonfunctional vitamin K dependent clotting factors as a cause of the observed coagulation disorder. The significant decrease of TT after 30 d in group 8_{60} has no pathological relevance and is likely due to accumulation of unused fibrinogen (factor I).

In both experiments, coagulopathy partially led to hemorrhages primarily in the gills and pseudobranchs. The hemorrhages caused a severe decrease of both the hematocrit value and the albumin level. In teleost fish, hematocrit values below 20 % indicate an anemia (Clauss et al., 2008). As described in literature for other organisms, anemia caused pale gills, discoloration, as well as behavioral changes in our study (Ludwig and Strasser, 2001; Rattner et al., 2014b). Erythrocytes are essential for the binding and transport of oxygen in vertebrates (Ludwig and Strasser, 2001). Interestingly, even in fish with severe anemia (hematocrit < 5 %), apathy but no dyspnea was observed. The high oxygen saturation in the flow-through system was probably sufficient for the relative low oxygen demand of these fish that were mostly inactive due to apathy. Anorexia and apathy in group 8_{60} (day 15–30) were related to a significantly reduced weight gain.

4.2. Brodifacoum residues in tissues

Our results implied that low doses of brodifacoum were bound almost completely in the liver of fish after gastrointestinal absorption into the blood stream (Table 3). Highest accumulation of SGARs in the liver compared to muscle and blood had also been shown in avian and mammalian species (Bachmann and Sullivan, 1983; Huckle et al., 1989, 1988). Liver brodifacoum concentrations in exposed trout from both experiments were dose-dependent and the non-linear concentration curve obtained from plotting measured hepatic concentration (wet weight) against the normalized cumulative dose administered fitted a second-order polynomial regression (Fig. A.2). A substantial decrease of brodifacoum liver concentration in fish over the course of the experiment was unlikely as brodifacoum showed no measurable hepatic *in vitro* clearance in rainbow trout (Regnery et al., 2022). Furthermore, in mammals, the resistance of brodifacoum to biotransformation and its persistence in liver (estimated half-life of > 300 d) is well documented, contrasting its more rapid elimination (several hours to multiple days) from blood (Horak et al., 2018 and references therein). However, ARs bind with high affinity to the blood transport protein albumin (André and Guillaume, 2004; Petitpas et al., 2001). Moreover, brodifacoum is assumed to undergo enterohepatic recirculation (Hauck, 2017; Horak et al., 2018). Elevated residual concentrations in the blood serum of trout exposed to the highest brodifacoum doses in the 15 d-experiment suggested that brodifacoum was particularly present in serum when liver tissue binding sites for 4-hydroxycoumarin anticoagulants were saturated (Mosterd and Thijssen, 1991; Thijssen and Baars, 1989). This can lead to increased hepatic concentrations as the liver is well supplied with blood, and remaining blood in the blood vessels is analyzed together with the hepatic tissue. On the contrary, reduced albumin levels due to blood loss will cause a loss of albumin-bound brodifacoum. This effect may partly explain the lower brodifacoum concentration detected in the liver of severe anemic fish of group 8_{60}, compared to group 6_{60} and 7_{60} at the second sampling. Furthermore, albumin is the most important protein to maintain the colloid oncotic pressure. An insufficient amount of albumin can lead to an excessive fluid loss from capillaries (Concannon, 1993). Given that brodifacoum concentrations were based on liver wet weight, a higher fluid content in the tissue will have an influence on calculated residue concentration, as described by Valverde et al. (2020) for bromadiolone concentrations in common kestrels (*Falco tinnunculus*).

4.3. Influence of housing conditions and multiple dosing

The study design of the two experiments differed in three main aspects: observation time (15 vs. 60 d), single vs. multiple administration of brodifacoum-spiked feed, and housing conditions (individual vs. groups). Exposure in groups compared to individual exposure had no effect on overt symptoms, mortality, and blood parameters during the first 15 d of the experiments. Therefore, a relatively uniform feed intake by all fish in a tank was assumed. Due to the chosen experimental design of the 60 d-experiment it is not possible to clearly separate the effects of exposure time and multiple feeding. Multiple feedings are known to especially influence the toxicity of FGARs in terrestrial species, whereas usually a single feed of SGARs delivers a toxic dose (Rattner and Mastrota, 2018; van den Brink et al., 2018). In the 60 d-experiment it was possible to analyze fish with the same cumulative dose of brodifacoum from treatment groups exposed to different multiple single doses at the first, second, and fourth sampling. Investigated effects of the same cumulative doses of brodifacoum were similar in the different treatment groups. Interestingly, the effect of the cumulative dose of 100 µg/kg bw brodifacoum on PT was slightly less pronounced in group 6_{60} after 60 d than in group 7_{60} and group 8_{60} after 30 d and 15 d, respectively. This might indicate some adaptive response to the chronic exposure of sublethal doses of brodifacoum. Though multiple feeding had no effect on corresponding hepatic concentration at lower total doses, we noticed that multiple feedings of high single doses of brodifacoum (i.e., 12.5, 25 and 50 µg/kg bw) led to more variable liver concentrations in fish compared to groups that received lower brodifacoum doses (Fig. 3). This increase of variation in hepatic residue concentrations at higher brodifacoum doses was also observed in the individually exposed trout in the 15 d-experiment (Fig. A.2). In good agreement with the above described pharmacokinetic of brodifacoum, hepatic concentrations were mostly driven by the total cumulative brodifacoum dose at sampling rather than a single administered dose, whereas serum concentrations in trout reflected the different administered single doses. Fish with the same cumulative dose revealed lower serum residues at sampling when total doses were administered in multiple smaller portions (Fig. 4).

4.4. Sensitivity of rainbow trout to brodifacoum

In the present study, coagulopathy was observed in rainbow trout ≥ 75 µg/kg bw and mortality in a range of 100–200 µg/kg bw brodifacoum (lowest observed adverse effect level – LOAEL). The corresponding no observed adverse effect level (NOAEL) in both experiments was ≤ 50 µg/kg bw brodifacoum. Compared to the LC_{50} of 40 µg/L provided in the European Union Competent Authority Assessment Report (eCA, 2016a), rainbow trout were less sensitive to brodifacoum when exposed via the diet. In the environment, however, a dietary exposure (e.g., via insects, poisoned carcasses, associated to particles) is more likely due to the hydrophobic properties of SGARs (Regnery et al., 2019a). Compared to the limited available data of other fish species, rainbow trout are very sensitive to brodifacoum. Riegerix et al. (2020) determined in four different fish species exposed via IP a median LD_{50} (72 h) range of 36,000 – 102,000 µg/kg bw brodifacoum. This considerable difference in sensitivity compared to our study might be due to the application method and the length of the observation period. The bioavailability and toxicity of substances can differ substantially between oral and IP application (Delistraty et al., 1998; Ning et al., 2015; Wang et al., 2015). Moreover, our results have shown that an observation period of 72 h is not sufficient to observe mortality caused by the AR-specific mode of action at concentrations of up to 10,000 µg brodifacoum/kg bw in rainbow trout. Thus, mortality observed in Riegerix et al. (2020) from 36,000 µg brodifacoum/kg bw could also be related to other factors, despite the observed coagulation disorder. In addition, the discrepancy in sensitivity could be caused by species-specific differences. Large species-specific variabilities in the susceptibility to SGAR are also known for birds (Thomas et al., 2011). Rattner and Mastrota (2018) reviewed the oral toxicity of ARs for mammals and birds. Rainbow trout are more sensitive to brodifacoum than most investigated bird species with an estimated LD_{50} of 250–11,600 µg/kg bw. In fact, in our study mortality of trout occurred at a dose comparable to that of barn owls (*Tyto alba*;

estimated lethal dose 150–182 μg/kg bw brodifacoum) – a species considered very sensitive towards SGAR intoxication – and sensitive mammals such as the Richardson's ground squirrel (*Spermophilus richardsonii;* LD_{50} estimate 130 μg/kg bw) and rabbits (*Oryctolagus cuniculus*; LD_{50} estimate 200–300 μg/kg bw) (Newton et al., 1990; Rattner and Mastrota, 2018).

4.5. Linking hepatic SGAR residues with effects in fish

The lowest mean hepatic brodifacoum concentration associated with effects was 122.6 ng/g (Table 3 and Table A.12). In livers of dead or moribund fish, residues between 71–826 ng/g brodifacoum were detected. These concentrations are within the range of previously detected environmental SGAR concentrations of almost 100 ng/g in livers of wild fish (Masuda, 2014; Regnery et al., 2024). When comparing hepatic AR residues detected in wild fish with the corresponding effects found in our study, it should be considered that the conditions during the experiments were designed to meet the requirements of rainbow trout to an optimal degree (OECD, 2019b). Furthermore, trout were prevented from other negative environmental stressors such as elevated water temperature, reduced dissolved oxygen levels, infectious diseases, predation, or contaminants. It is long recognized that captive birds may be less sensitive to toxicity of ARs than free-ranging individuals (Rattner et al., 2020). In the environment, adverse effects in fish induced by the SGAR-dependent coagulation disorder are likely to occur at even lower hepatic concentrations than reported here. The considerable time-delay of 17 days between brodifacoum exposure and onset of mortality resulted most likely in overdosing of the test fish fed every 7 or 8 days with brodifacoum-spiked feed.

5. Conclusion

To assess the environmental impact of rodenticide residues measured in wild fish, we exposed rainbow trout to brodifacoum-spiked feed. Brodifacoum caused coagulopathy, anemia, and mortality in rainbow trout at environmentally relevant hepatic concentrations. The presented data provide a solid reference for critical hepatic SGAR residues in fish and thereby help interpreting fish monitoring data in terms of potential adverse effects. The observed effects were dose-dependent and consistent with the mode of action known for other vertebrates. In comparison with other non-target organisms, rainbow trout are relatively sensitive to brodifacoum exposure. Depending on the species, the observation period is crucial for the assessment of the impact of brodifacoum on fish: after uptake of brodifacoum-spiked feed, the onset of overt symptoms took at least 14 d in rainbow trout. In both experiments, even the lowest tested brodifacoum dose led to detectable hepatic residues. Accordingly, fish exposed to SGARs are a potential source for secondary poisoning of piscivorous predators in the aquatic food chain. Considering the huge species-specific differences in susceptibility to AR intoxication and the very limited data on fish, further studies on the lethal and sublethal effects of different AR in fish are necessary. Overall, our study clearly indicates the risks associated with the use of AR for wild fish and reinforces the need to stipulate all available and appropriate risk mitigation measures to prevent emissions at their source, e.g., from urban rat baiting campaigns in sewers and near watercourses, or during rat eradication measures on islands and coast lines.

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Hermann Ferling: Writing – review & editing, Methodology, Investigation, Data curation. **Karina Annika Bucher:** Writing – review & editing, Methodology, Investigation. **Stefanie Jacob:** Writing – review & editing, Supervision, Conceptualization. **Julia Regnery:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Conceptualization. **Hannah Schrader:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **Julia Schwaiger:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Anton Friesen:** Writing – review & editing, Supervision, Conceptualization. **Hannah Schmieg:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117629.

Data availability

Data will be made available on request.

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SenesTech and Irvine Campus Housing Authority Announce Successful Field Trial of Evolve™ Rat Birth Control at UC Irvine

SURPRISE, Ariz., June 25, 2025 – SenesTech, Inc. (NASDAQ: SNES, "SenesTech" or the "Company"), a leader in fertility control for managing animal pest populations, today announce a nine-month field trial of Evolve™ Rat Birth Control at the University Hills (UHills) residential community at the University of California, Irvine. The project was conducted in partnership with the Irvine Campus Housing Authority (ICHA), the nonprofit corporation that manages University Hills, and reflects a growing commitment to ecologically responsible pest management.

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The initiative began in September 2024 and marked the exclusive use of Evolve Rat Birth Control across common areas of the UHills community. This deployment was part of a broader effort supported by residents interested in moving away from rodenticides in favor of integrated pest management (IPM) strategies that prioritize environmental safety and wildlife preservation. Located adjacent to a wildlife reserve, the community's shift away from rodenticides further supports the protection of local wildlife and ecosystems.

"This project highlights how community-driven action and science-based solutions can work together to support sustainability," said Joel Fruendt, President and CEO of SenesTech. "Evolve aligns with these goals by providing a non-lethal, poison-free solution to rodent control."

Evolve was deployed across 267 bait stations previously containing rodenticides and snap traps. According to field observations, rats consumed 98% of Evolve across all stations each month, indicating high acceptance and performance of the bait. As expected, consumption dropped as the rat population dropped.

The field trial also supported the efforts of the HOOT Group (Help Our Owls Thrive), a grassroots initiative formed by UHills residents to promote owl nesting and reduce rodenticide use. Together, the partners implemented a range of actions, including installation of enclosed snap traps, removal of rodent harborage areas, and installation of barn owl nesting boxes to encourage natural predation.

"The partnering with SenesTech in utilizing Evolve has supported ICHA's and the UHills community's heightened focus on IPM sensitivity to the local wildlife and raptor population," said Andrew Herndon, Vice President of Community Development at ICHA. "Evolve appears to offer a reduction of poisons in the environment while at the same time a reduction of the rodent population."

As a result of the collaboration, rodenticides have been eliminated from all 267 bait stations across common areas in UHills. SenesTech is pleased to contribute to this transition and to support communities like UHills and organizations like ICHA and HOOT that are leading the way in redefining effective, responsible rodent control.

ICHA plans to continue using Evolve in University Hills.

About SenesTech

We are committed to improving the health of the world by humanely managing animal pest populations through our expertise in fertility control. We invented ContraPest®, the only U.S. EPA-registered contraceptive for male and female rats, as well as Evolve™ Rat and Evolve™ Mouse, EPA-designated minimum risk contraceptives for rodents, reflecting our mission to provide products that are proactive, safe and sustainable. ContraPest and Evolve fit seamlessly into all integrated pest management programs, significantly improving the overall goal of effective pest management. We strive for clean cities, efficient businesses and happy households – with a product designed to be humane, effective and sustainable.

For more information visit https://senestech.com/.

Safe Harbor Statement

This press release contains "forward-looking statements" within the meaning of federal securities laws, and we intend that such forward-looking statements be subject to the safe harbor created thereby. Such forward-looking statements include, among others, our belief that the UHills project reflects a growing commitment to ecologically responsible pest management; our belief that this

project highlights how community-driven action and science-based solutions can work together to support sustainability; and our expectation for a multi-pallet restocking order for continued deployment. Forward-looking statements may describe future expectations, plans, results or strategies and are often, but not always, made through the use of words such as “believe,” “may,” “future,” “plan,” “will,” “should,” “expect,” “anticipate,” “eventually,” “project,” “estimate,” “continuing,” “intend” and similar words or phrases. You are cautioned that such statements are subject to risks, uncertainties and other factors that could cause actual results to differ materially from those reflected by such forward-looking statements. Such factors include, among others, the successful commercialization of our products, market acceptance of our products, regulatory approval and regulation of our products and other factors and risks identified from time to time in our filings with the Securities and Exchange Commission, including our Annual Report on Form 10-K for the fiscal year ended December 31, 2024. All forward-looking statements contained in this press release speak only as of the date on which they were made and are based on management's assumptions and estimates as of such date. Except as required by law, we do not undertake any obligation to publicly update any forward-looking statements, whether as a result of the receipt of new information, the occurrence of future events or otherwise.

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SenesTech, Inc. represents that Evolve™ qualifies as a minimum risk pesticide under FIFRA Section 25(b) and is exempt from registration by the U.S. Environmental Protection Agency.

ContraPest® is registered federally as a General Use Product but requires an RUP license if being deployed in Connecticut. Due to conditional registration status in California, the product is not allowed to be used in public schools or childcare facilities within the state of California at this time.

ContraPest® is a registered trademark of SenesTech, Inc. Rat Birth Control™, Elevate Bait System™, Elevate Bait Station™, ContraPest Elevate Bait™, Isolate Bait System™, and Evolve™ are trademarks of SenesTech, Inc. ContraPest is intended for professional use only. Read and follow all label instructions for target species Norway and roof rats.

ContraPest 400mL tanks and trays are designed to fit the **PROTECTA EVO Express® bait station. PROTECTA EVO Express is a registered trademark of Bell Laboratories, Inc. and SenesTech is not related or affiliated with Bell Laboratories, Inc.

Limit of one discount or promotion per transaction.

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Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study

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Abstract

Anticoagulant rodenticides (ARs) are increasingly recognized as a threat to nontarget wildlife. High exposure to ARs has been documented globally in nontarget predatory species and linked to the high prevalence of an ectoparasitic disease, notoedric mange. In southern California, mange associated with AR exposure has been the proximate cause of a bobcat (Lynx rufus) population decline. We measured AR exposure in bobcats from two areas in southern California, examining seasonal, demographic and spatial risk factors across landscapes including natural and urbanized areas. The long-term study included bobcats sampled over a 16-year period (1997-2012) and a wide geographic area. We sampled blood (N = 206) and liver (N = 172) to examine exposure ante- and post-mortem. We detected high exposure prevalence (89 %, liver; 39 %, blood) and for individuals with paired liver and blood data (N = 64), 92 % were exposed. Moreover, the animals with the most complete sampling were exposed most frequently to three or more compounds. Toxicant exposure was associated with commercial, residential, and agricultural development. Bobcats of both sexes and age classes were found to be at high risk of exposure, and we documented fetal transfer of multiple ARs. We found a strong association between certain levels of exposure (ppm), and between multiple AR exposure events, and notoedric mange. AR exposure was prevalent throughout both regions sampled and throughout the 16-year time period in the long-term study. ARs pose a substantial threat to bobcats, and likely other mammalian and avian predators, living at the urban-wildland interface.

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Laboratory Evaluation of the Effectiveness of the Fertility Control Bait ContraPest® on Wild-Captured Black Rats (*Rattus rattus*)

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Robert T. Sugihara

Israel Leinbach

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ABSTRACT: A non-toxic liquid fertility control bait for rats has recently become commercially available (ContraPest® from SenesTech, Inc.). This product contains two chemicals, both of which impair spermatogenesis in male and reduce ovulations in female rats. We tested the efficacy of this bait in wild-caught adult black rats from the island of Hawai'i in a short-term laboratory trial. A control group (n = 25) was offered placebo bait and the treatment group (n = 25) was offered fertility control bait, both *ad libitum*, during a 15-day introduction period and during the first of four breeding rounds, for a total of 58 days of exposure. After treatment, all rats were provided placebo bait for the remainder of the study and randomly paired with mates from within their treatment groups for two additional breeding cycles. Treatment and control groups comprised 10 breeding pairs each, with random re-pairings between breeding rounds. The treatment group produced no litters during the first and second breeding rounds, while 70% of the control females produced litters. In the third breeding round, 70 days after stopping treatment, the treatment group produced three litters (six pups) compared to seven litters (24 pups) in the control group. During a fourth and final breeding round, control rats were crossed with treated rats, producing six litters (27 pups) from treated dams and nine litters (40 pups) from control dams, indicating no apparent infertility effect 99 days after cessation of treatment. This study demonstrates that the reproduction rate of wild-caught black rats can be chemically suppressed if provided *ad libitum* access to the fertility control bait under laboratory conditions.

KEY WORDS: 4-vinylcyclohexene diepoxide, black rat, contraception, fertility, laboratory efficacy, *Rattus rattus*, reproductive inhibition, triptolide, VCD

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INTRODUCTION

Invasive mammals impose great environmental and economic costs through damage to agricultural crops, natural resources, and human health and safety (Bergman et al. 2000, Pimentel et al. 2005). Invasive mammals can be particularly devastating on islands and have led to considerable species decline and extinctions (Courchamp et al. 2003, Doherty et al. 2016), with rats being one of the most damaging taxa of invader (Jones et al. 2008, Varnham 2010). In addition to the damage they cause in natural environments, invasive pest rodents have caused severe human and economic costs through damage to agriculture contributing to famine, vectoring zoonotic diseases, and mechanical damage resulting from gnawing and nesting behavior (Singleton et al. 2010, Himsworth et al. 2013). In Hawai'i, invasive black rat (*Rattus rattus*) populations damage crops and food stores, kill native flora and fauna, and are reservoirs and vectors of human disease (Meerburg et al. 2009, Shiels et al. 2014), including leptospirosis and rat lungworm disease (Jarvi et al. 2014, 2015, 2017).

In addition to traditional components of integrated pest management such as sanitation (removal of food sources), habitat management, and physical exclusion, large-scale rat control in protection of agriculture, human heath, and natural resources has typically involved the use of rodenticides: lethal toxicants formulated into an attractive and palatable bait matrix (Hadler and Buckle 1992, Buckle 1999, Witmer et al. 2007, Witmer and Eisemann 2007, Buckle and Smith 2015). However, shifting societal values (e.g., in opposition to perceived risk of poisoning of nontarget wildlife, animals and children) are increasing the demand for non-toxic, non-lethal alternatives for resolution of human-wildlife conflicts. Traditional anticoagulant rodenticides have aroused concern over poisoning of non-target species, environmental contamination, and humaneness (Mason and Littin 2003, Eason et al. 2010). Wildlife fertility control has been considered as a potential long-term management approach for reducing pest populations and the damage they cause (Miller at al. 1998). Fertility control has been predicted to prevent the rebound of rodent populations seen after rodenticide application (Gao and Short 1993) by reducing the rate of reproduction following temporary release from density-dependent population regulation (Jacob et al. 2008).

SenesTech, Inc. (Flagstaff, Arizona) markets a U.S. Environmental Protection Agency-registered, commercial liquid bait formulation, ContraPest®, for fertility control in rats. It contains two active ingredients that target both follicle development and spermatogenesis, blocking reproduction in both sexes. The active ingredient 4-vinylcyclohexene diepoxide (VCD) causes primordial follicle depletion leading to premature ovarian failure (Hoyer et al. 2001, Mayer et al. 2002, Mayer et al. 2004, Mark-Kappeler 2011). Follicular maturation progresses from the primordial stage to primary, secondary, antral and preovulatory in preparation for ovulation (Mayer et al. 2002). VCD targets the finite pool of primordial follicles;

once depleted, and after the remaining follicle types have been eliminated by atresia or ovulation, ovarian function is terminated (Hoyer et al. 2001, Mayer et al. 2004, Jacob et al. 2008, Mauldin 2013). VCD causes primordial follicle loss by interfering with KIT signaling, a key cellular growth and survival pathway within the oocyte (Mark-Kappeler et al. 2011). Atresia is a natural process in the ovary to eliminate follicles not destined for ovulation. VCD greatly accelerates this natural process (Hoyer at al. 2001). The second active ingredient in ContraPest is triptolide, a diterpene triepoxide purified from the traditional Chinese medicinal plant *Tripterygium wilfordii*. Triptolide stops growing follicles in the ovary and sperm production in the testes (Lue et al. 1998, Huynh et al. 2000, Xiong et al. 2011, Zeng et al. 2017). ContraPest has very low concentrations of both actives, VCD at 0.09% and triptolide at 0.001%. The combination of these two active ingredients acts synergistically to suppress reproduction in both sexes. Witmer et al. (2017) recently tested the palatability and efficacy of ContraPest fertility control bait in both Sprague-Dawley laboratory rats and in wild-caught Norway rats (*R. norvegicus*). Sprague-Dawley rats were provided *ad libitum* access to the liquid bait, along with *ad libitum* chow and water for 21 days. Rats that took treatment bait were placed in breeding pairs, as were control rats that took bait without active ingredients. Rats that received treatment bait had no offspring, while 100% of control rats had litters after one breeding round. Similar results of no offspring were found in breeding pairs of wild-caught Norway rats, tested in the laboratory, which took treatment bait and then completed two breeding rounds (Witmer et al. 2017).

ContraPest has yet to be tested on black rats, the species with the most widespread impacts on island ecosystems (Jones et al. 2008, Shiels et al. 2014). To date, there are no reports on the impact of the combination of ContraPest's two active ingredients on black rats, nor has the impact of VCD on their fertility been reported. Of the two active ingredients, only triptolide's impact on male black rat fertility has been studied (Singla et al. 2013, Singla and Challana 2014). In Singla and Challana (2014), the reproductive toxicity of triptolide was examined in no choice feeding at 0.1%, 0.2%, or 0.3% for 5 days with wild-caught male black rats. After 15 days post-dosing, the treated male rats were mated with healthy, untreated and cyclic female rats for 15 days. Only the male rats that took 0.2% triptolide sired no pups which may have been due to significantly reduced sperm motility and viability (Singla and Challana 2014). When the concentrations of triptolide taken by wild-caught brown rats versus wild-caught black rats are compared, taking into account the difference in percent concentrations in the two different baits, average consumption for each male rat, and difference in body weights between the larger brown versus black rats, the male black rats were exposed to 1,376 times the amount of triptolide taken by choice by the male brown rats. No mortalities were reported for either black or brown male rats in these two studies (Dyer et al. 2013, Singla and Challana 2014). Given the large difference in triptolide concentration that induced infertility in male black versus male brown rats, we sought to determine what impact ContraPest would have on the fertility of wild-caught black rats. Perhaps ContraPest would be ineffective in black rats due to their apparent insensitivity to triptolide, as over a 1000-fold greater triptolide was needed to suppress male rat fertility. Because ContraPest is presented in bait stations which both male and female black rats would visit, we presented ContraPest to both sexes to determine the impact on their fertility and fecundity.

METHODS

Animal Acquisition, Preparation, and Disposition

Wild black rats were live-trapped in forests and other conservation areas near Hilo and Volcano, County of Hawaii. In addition to trapping at our own USDA Hilo facility, permissions for trapping were granted by the Environmental Office of the Keaukaha Military Reservation, the site manager of Mauna Loa Orchards, and private owners of residential properties in the village of Volcano. Captured rats were transported to the testing facility and dusted with Drione® insecticide (Bayer, Research Triangle Park, NC) to treat for ectoparasites before being housed. Fifty rats of equal sex ratio were housed individually in numbered metal laboratory cages in a climate- and lighting-controlled laboratory space at the testing facility (20-22°C, ambient humidity, and 12 hr on/off light cycle). Cages (22 cm × 57 cm × 19 cm) were furnished with PVC refuge tubes sized for one or two rats (isolation or breeding events) and commercially purchased shredded paper bedding, replaced as needed. All rats had unrestricted access to a maintenance diet of Purina® rodent chow pellets (Nestle Purina PetCare Company, St. Louis, MO), and water was provided *ad libitum* in 250-ml inverted glass bottles with stainless steel sipper tubes throughout the duration of the study. Rats also received wood chew sticks with replacement as necessary.

All rats were individually housed for a minimum quarantine period of 3.5 weeks to ensure that no females were pregnant at the outset of the study phase. Rats were weighed at the beginning of the quarantine period, prior to pairing, and again at the end of the trial phase. All young born during the study were removed upon parturition and euthanized via an overdose of inhalant anesthesia (isoflurane) with subsequent carbon dioxide (CO_2) immersion. Adult rats were euthanized via CO_2 overdose at the end of the study. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and within the terms of the study protocol approved by the Institutional Animal Care and Use Committee of the U.S. Department of Agriculture National Wildlife Research Center (QA-2570).

Statistical Analyses

All statistical analyses and data visualization were performed in the R language for statistical computing (R Core Team 2016). Specific functions and tests are described within the methods subsections below.

Bait Consumption

Liquid ContraPest, containing the active ingredients VCD and triptolide (i.e., active bait), or an identical formulation lacking the active ingredients (i.e., placebo bait), was offered *ad libitum* in identical 250-ml inverted

glass bottles, as were the bottles providing water. Daily bait consumption was estimated by measuring the bait level within the bottles with a graduated scale. While co-housed for breeding we continued to record bait consumption, though we were unable to determine how much bait was consumed by each individual. To test for an effect of the active ingredients on bait consumption (i.e., palatability effects of VCD and triptolide), data from the initial exposure and first breeding cycle phases (the period during which the treatment group received the active bait) were subjected to a linear mixed effects model with bait type (active vs. placebo), sex, and study phase (active bait exposure vs. pre- and post-exposure periods) as fixed effects and individual identification (ID) as a random effect (1|ID) to account for multiple repeated measures for each individual. Modeling was conducted using the function "lmer" in the package "lme4" (Bates et al. 2015), with the model specified as:

$$\text{consumption} \approx \text{bait} + \text{sex} + \text{phase} + (1|\text{ID})$$

To obtain a p-value for the effect of bait type on consumption, we performed a likelihood ratio test comparing this model to a null model without the bait term in an analysis of variance (ANOVA); the p-value for the χ^2 comparison of the two models is reported as the statistical significance of the bait effect.

Reproductive Inhibition Trials

Prior to pairing for breeding, all rats were pretreated with the placebo bait formulation for a five-day conditioning period to ensure that rats were familiar with the bait prior to the treatment period (trial Days -6 to -1). Within each sex group, 13 rats were randomly assigned to the active bait treatment group and 11 females and 13 males were assigned to the placebo bait control group. After the conditioning period, the treatment group was administered the active bait for 15 days while the control group continued to receive the placebo bait (Days 0 to 15). Weight, sex, cage number, and treatment group assignment of each pair was recorded before the initiation of the breeding cycles.

During the first of four breeding rounds (Round 1, Days 15 to 35), the treatment and control groups continued receiving active and placebo bait, respectively. Ten females were randomly paired with ten males within their respective study groups (treated females paired with treated males, control females paired with control males) and the males were placed within the females' cages for mating, with individual IDs recorded for each pairing. The remaining rats in each group continued to be housed individually, to be substituted for rats found to be unfit for breeding due to poor condition, injury, or rejection of a male by the female partner during the course of the breeding cycle.

Males were paired with females for 21 to 23 days. If a male was rejected by the female within 24 to 48 h, one of the spare males from the same study group (treatment or control) was substituted for breeding. Females and/or cage papers were examined daily for discarded vaginal plugs as an indication that they had been inseminated. After the pairing period, males were removed and returned to their individual cages. Females were monitored for parturition daily for 23-28 days following removal of males. Within 24 h of birth, pups were removed, counted, and euthanized.

At the completion of the first breeding cycle, the active bait provided to the treatment group was withdrawn and replaced with the placebo bait to determine the persistence of a reproductive inhibition effect (Day 58). At this time, the treatment group had been continuously exposed to the active bait for 58 days. All rats were provided the placebo bait for the remainder of the study.

For a second and third breeding cycle (Rounds 2 and 3, Days 58 to 79 and 105 to 127), pairings within study groups were re-randomized without replacement so that males were placed with different females than in previous breeding cycles. For a fourth and final breeding cycle (Round 4, Days 156 to 177), females from the treatment group were crossbred with males from the untreated control group, and treated males were paired with un-treated females in order to assess whether treatment of a single sex suppressed reproduction.

After the last round of breeding, all animals were euthanized and body weights recorded. Liver, kidneys (combined), spleen, adrenal glands (combined), and reproductive organs were excised, cleaned of fats and/or connective tissues, and weighed for future comparative analysis.

Statistical differences between counts of litters for treatment and control groups, per breeding round, were tested with Fisher's exact tests Wilcoxon rank tests, with α set at 0.05 and two-tailed p-values reported. These methods were not intended to distinguish between a contraceptive effect on males or females.

RESULTS

Bait Consumption

Daily bait consumption by sex, study group, and study phase is depicted in Figure 1. All rats readily consumed the bait. The median rat (ranked by consumption) consumed an average of 74 ml of bait per day; individual means ranged from 66 to 83 ml per day. There was no effect of inclusion of the active ingredients in the bait formulation on bait consumption (i.e., there was no apparent negative effect of active ingredients on palatability; study phase term $p = 0.739$).

During this project, males (which grow larger than females) gained significant weight, but there was no discernible difference in weights between test groups of males or females receiving placebo or active-ingredient baits during the treatment phase (Figure 2).

Reproductive Inhibition Trials

During pairings, there were only two occasions when males were removed and replaced due to incompatibility/ aggression by females. Pairings and litter size details for all four breeding rounds are tabulated in Siers et al. (2017).

Numbers of litters and pups per litter for each of the ten breeding pairs per round are summarized in Table 1. During the first breeding round, when the treatment group had been exposed to active ContraPest bait for 15 days and was continuing to consume the active bait, there were no litters within the treatment group, while seven litters, totaling 32 pups, were born to the 10 control pairs (70% breeding success). During the second breeding round, which began simultaneously with the replacement of the

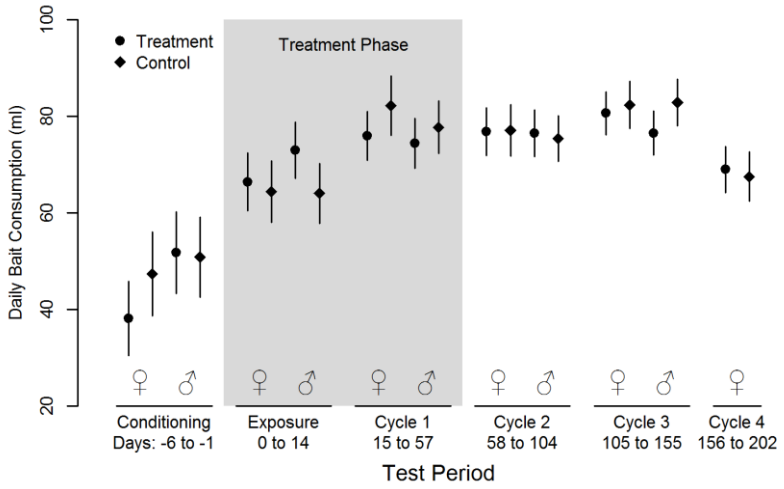

Figure 1. Bait consumption. Mean daily bait consumption and 95% confidence intervals (1.96*SE) by sex, study group, and study phase. Bait including the active ingredient was only offered during the Treatment Phase. Individual consumption data were only available while rats were individually-house (not while paired for breeding). There is no Cycle 4 consumption data for males because they were euthanized immediately following breeding. N = 11 for the female control group, N = 13 for all other groups.

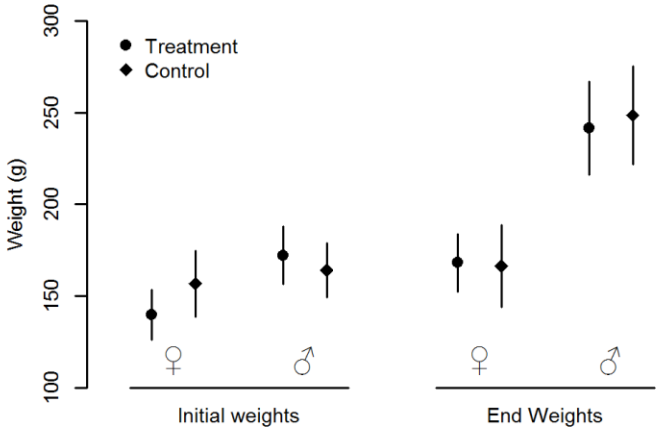

Figure 2. Body weights. Mean rat body weights and 95% confidence intervals (1.96*SE) by sex and study group. Within sexes, there were no significant weight differences between treatment and control groups (α = 0.05). N = 11 for the female control group and N = 13 for all other groups.

Table 1. Litter count and litter size results for female rats (N = 10 per study group). "Bait" indicates whether the treatment group was provided either the active ingredient ContraPest product or the placebo version during the breeding cycle. "Mating" denotes whether females were matched to males by study group (treatment-treatment/control-control) or crossed with males of the opposite study group (treatment-control/control-treatment). "Mean Litter" size is calculated only from females with litters (zeroes not included in the average); however, the Wilcoxon rank test for difference in litter size is based on a litter size of zero for dams without litters. * p ≤ 0.05; ** p ≤ 0.01.

Breeding Round	Days Paired	Bait	Mating	Litters		Pups		Mean Litter ± SD	
				Ctrl	Trt	Ctrl	Trt	Ctrl	Trt
1	15-35	Active	Matched	7	0**	32	0	4.57 ± 1.05	0**
2	58-79	Placebo	Matched	7	0**	27	0	3.86 ± 2.17	0**
3	105-127	Placebo	Matched	7	3	24	6	3.43 ± 1.18	2.00 ± 1.41*
4	156-177	Placebo	Crossed	9	6	40	27	4.44 ± 2.91	4.50 ± 2.22

treatment group's active bait with placebo, there continued to be no reproduction in the treatment group pairs and 70% breeding success (seven litters, 27 pups) in the control group. During both first and second breeding rounds, the difference between seven control litters and zero treatment litters was statistically significant ($p = 0.004$). By the beginning of the Round 3, the active bait had been replaced by placebo for 47 days. During this round, control group reproduction remained at 70%, while treatment group reproduction increased to 30%; though continued reproductive suppression is apparent, the difference between control and treatment group litters was not statistically significant at $\alpha = 0.05$ ($p = 0.178$). Considering only females that produced litters, the litter sizes in the treatment group (four, one, and one) were smaller than those in the control group ($p \leq 0.05$). By the fourth round of breeding, commencing 99 days after removing active bait, treatment group females paired with control group males reproduced at a rate indistinguishable from control group fecundity in previous rounds. There was no apparent suppressive effect when mating control group females with treatment group males, with this group producing the most litters (90% breeding success) and bearing litter sizes indistinguishable from previous control female breeding rounds.

DISCUSSION

These results demonstrate complete reproductive inhibition for wild-caught black rats exposed to ContraPest bait containing the active ingredients VCD and triptolide, *ad libitum*, under laboratory conditions, for at least 15 consecutive days prior to mating and throughout a 43-day breeding cycle. The inhibitive effect persisted through the second breeding cycle. When paired a third time, 47 days after cessation of treatment, a partial suppressive effect was apparent but not statistically significant, though litter sizes were significantly smaller for the few treatment females that did reproduce. By 99 days post-treatment (Round 4) there was no apparent effect of reproductive inhibition. Given that fertility was rebounding by the third breeding cycle, we are unable to draw any useful inference from the cross-breeding of treated and control animals, and the ability to detect any potential sex-specific effect is confounded by the dissipation of the treatment effect. The impact of the two active ingredients of ContraPest caused infertility for a length of time that exceeded the spermatogenic cycle reported by Singla et al. (2013); however, our study design did not allow for discrimination between a contraceptive effect on males or females. In practice, both sexes will receive the bait, and based on prior research cited in the introduction we expect that both sexes are affected to some degree.

Reproduction within our control group was not 100%. During the first three breeding cycles, three (30%) of the control group pairings did not resulted in litters, and one (10%) of the parings in the fourth cycle did not produce a litter. This could potentially be a result of failure of wild-caught rats to fully adjust to captivity in a relatively short timeframe, or an indication of incomplete fertility in the source population from which these individuals were drawn. While differences in litters and litter sizes were statistically significant during the first two breeding rounds, without 100% reproduction in the control group it is difficult to argue that the lack of any litters in the first two breeding rounds was solely attributable to the ContraPest treatment and with no influence of sub-fertility in the treatment group.

By design, ContraPest is a contraceptive and not a sterilant. As a result, no evidence of permanent infertility was detected following the 58-day active bait exposure period. Whether more prolonged exposure to ContraPest would lead to permanent sterilization cannot be inferred from our study. Further studies would be needed to assess the effect of long-term exposure on fertility.

Refinements of this or other fertility control baits might afford non-toxic and non-lethal alternatives for protection of agriculture, human health and safety, and natural resources under some management scenarios. For instance, ContraPest could complement conventional use of toxicants that have caused a rapid knockdown of the pest population; following up with the use of ContraPest could prevent the well-known rebound of a poisoned rat population (Andrews 1977). Timing the use of ContraPest would be key to diminishing rat population expansion due to seasonal weather changes and/or abundance of food sources. In Hawai‘i, for example, ContraPest could be considered to suppress the marked increase in rat population that follows the peak of strawberry guava fruiting by two to three months (Shiels 2010).

ContraPest has also undergone successful trials for reducing brown rat population levels in the New York City Subway system in 2013 (Klein 2017). Because brown rats in subway trash rooms chose liquid when given choice between solid and liquid matrices to deliver the active ingredients, ContraPest is currently formulated as a liquid. For treatment of black rats in challenging tropical terrains, where deployment of bait boxes with liquid tanks is not feasible, it will be necessary to develop and evaluate a solid form of ContraPest that can be aerially deployed and is durable in the tropical environment. This study demonstrates that the active ingredients in ContraPest, when ingested *ad libitum* in the current liquid formulation, can profoundly reduce black rat reproductive output.

ACKNOWLEDGEMENTS

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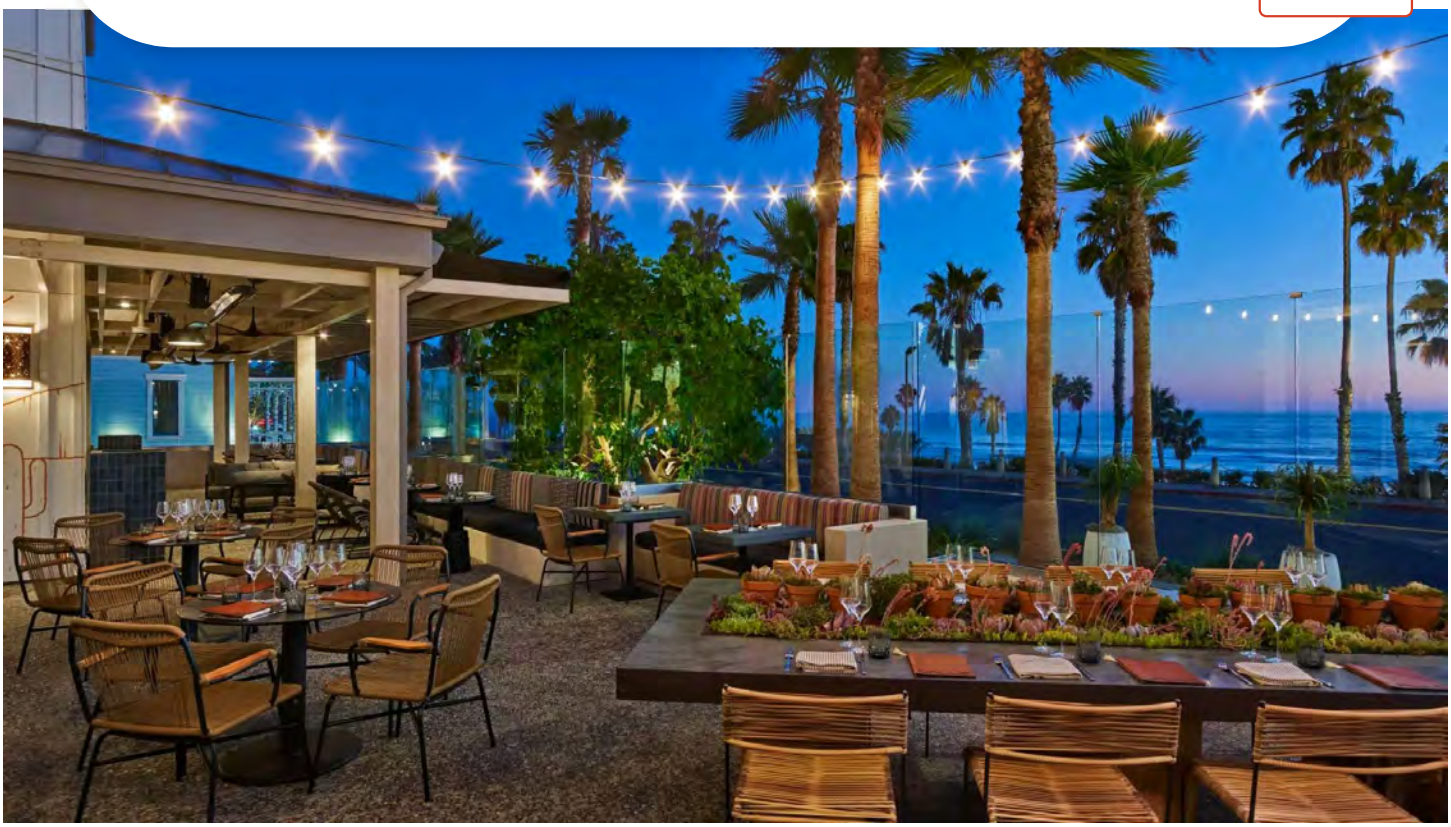


How Many Restaurants Are In California – Report

Thomas Campbell

Updated March 17, 2025

There are **98,156 restaurants in the state of California** according to data which we compiled using Google's Maps Data. This number represents all restaurants listed on Google Maps in California and includes bars, coffee shops and other restaurant categories.

The data in this article is a representation of the number of restaurants in California and the various key findings we

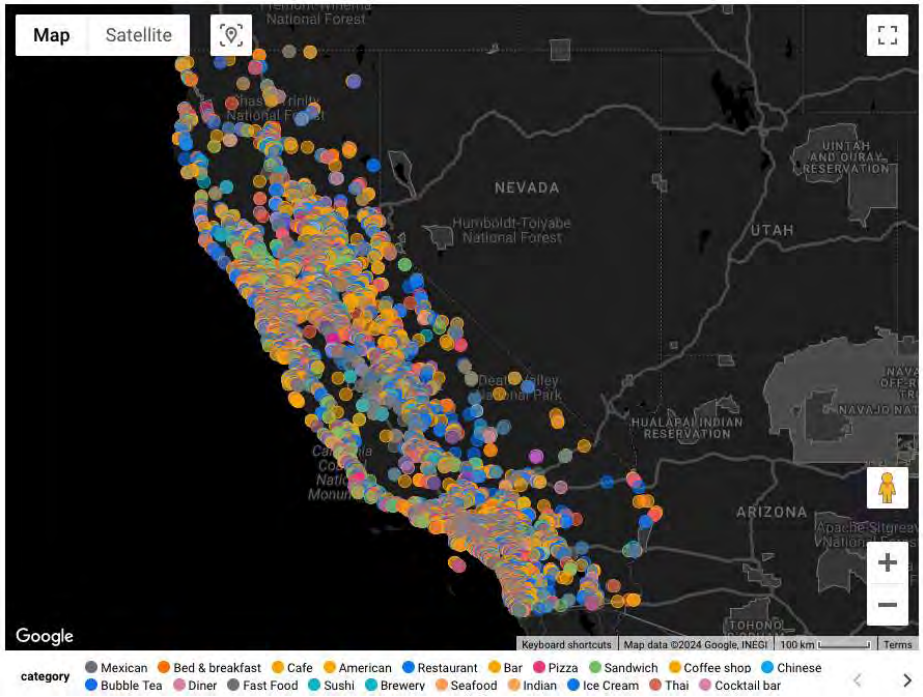
Table of Contents

discovered.

Bel
vie
Los

98,156 Restaurants in California

There are 98,156 restaurants in the the state of California according to Google's data. The most popular restaurant categories (in no particular order) in California include: Mexican, Cafe, American, Pizza, Bar, Sandwich, Chinese, Bubble Tea, Fast Food and others.



7.6 Restaurants Per Capita

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Restaurants Per 1000 People

Los Angeles Has The Most Restaurants

Los Angeles has the most restaurants in California with 6,682. San Diego is not far behind Los Angeles and has 3,695 total restaurants while San Francisco has 3,234. Rounding up the top five are San Jose with 2,158 and Fresno with 1,403.

3.	CA	San Franci...	3,234
4.	CA	San Jose	2,158
5.	CA	Fresno	1,403
6.	CA	Oakland	1,282
7.	CA	Long Beach	1,276
8.	CA	Sacramento	1,199
9.	CA	Bakersfield	1,122
10	CA	Riverside	852

0.63 Restaurants Per Square Mile

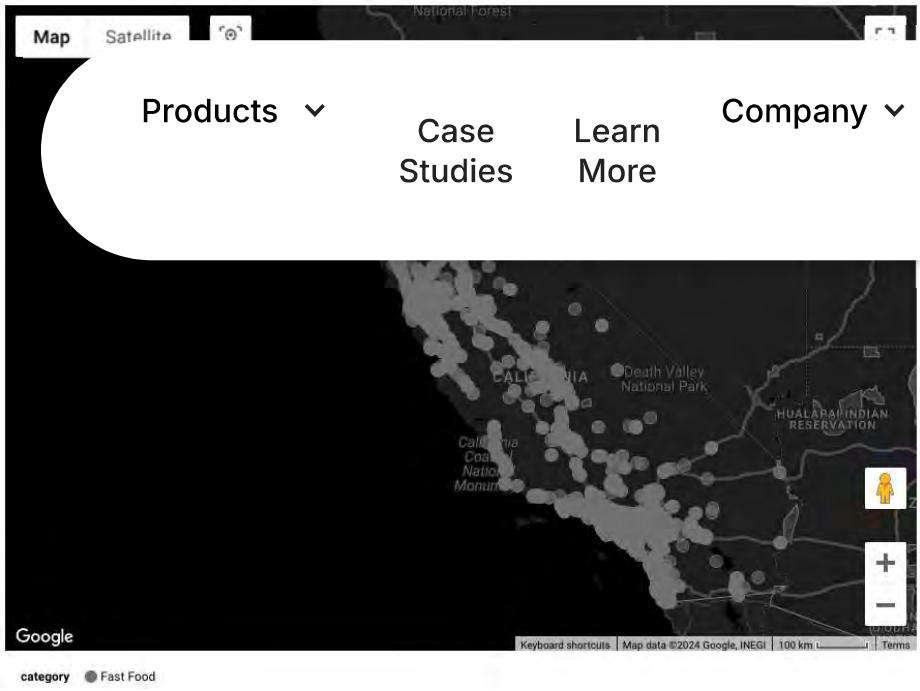
With land area of 155,812.8 square miles according to United States Census Bureau, there are 0.63 restaurants per one square mile in California.

0

Restaurants Per Square Mile

4,596 Fast-food Restaurants

4,596 restaurants are fast food which makes up about 5% of all restaurants in California. Most popular chains include McDonald's, Carl's Jr., Wendy's and others.



Fast Food Price Dissatisfaction Up 31%

Due to California's new minimum wage state law, we also tracked Google Maps reviews for the keyword 'expensive' in fast-food restaurant listings from November, 2023 up until now.

Prior to the minimum wage increase for fast food workers coming into effect, there was an average of 1,332 Google reviews that contained the keyword word 'expensive' for fast food restaurants. After the law came into effect, there was an average of 1,740 Google reviews for fast-food restaurants that had the keyword 'expensive' in it. This is an increase of 31% since the law came into effect.

+0%

More Fast Food Restaurant 'Expensive' Reviews

Among a variety of cuisine options throughout California, Mexican cuisine has the highest number of restaurants with 14,457. This is followed by the following cuisine focused restaurants: Chinese (3,532), American (2,683), Japanese (1,905), Italian (1,772) and Indian (986).

88 Michelin Star Restaurants

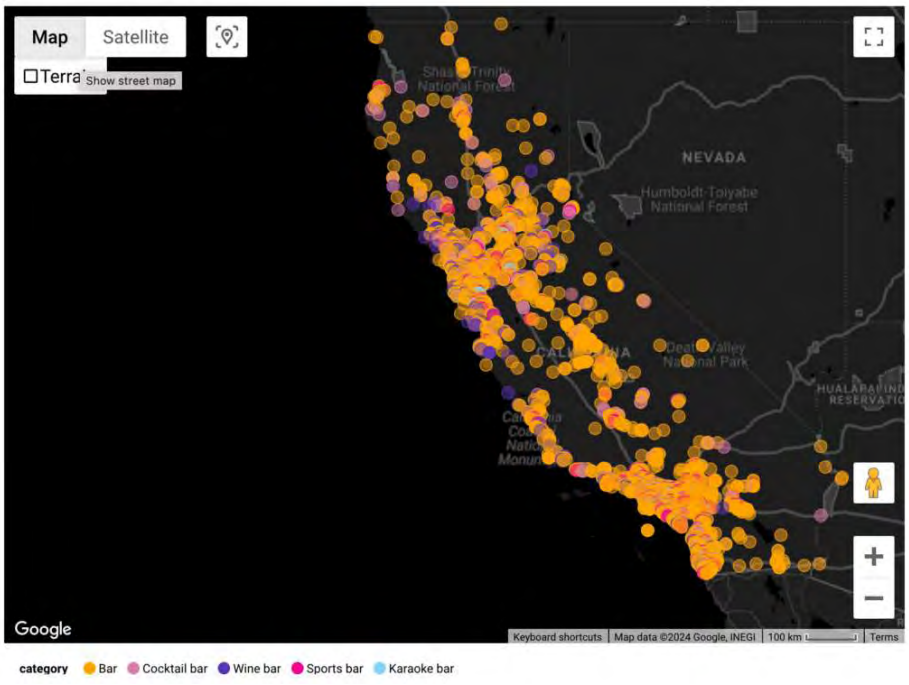
California is home to 88 Michelin Star restaurants that range from one to three stars. 66 restaurants in California have one Michelin star distinction, 12 have two Michelin star distinction and six have three star distinction. The six restaurants with three Michelin star distinction are: Atelier Crenn (San Francisco), Addison (San Diego), Quince (San

Francisco), Single Thread (Healdsburg), Benu (San Fran

Michelin Star Restaurants

6,254 Bars

California has a lot of bar options throughout the state which includes 6,254 options. Popular bar categories include: bar, sports bar and cocktail bar.



13,547 Pizza Restaurants

California has plenty of pizza restaurants with 13,547 resta
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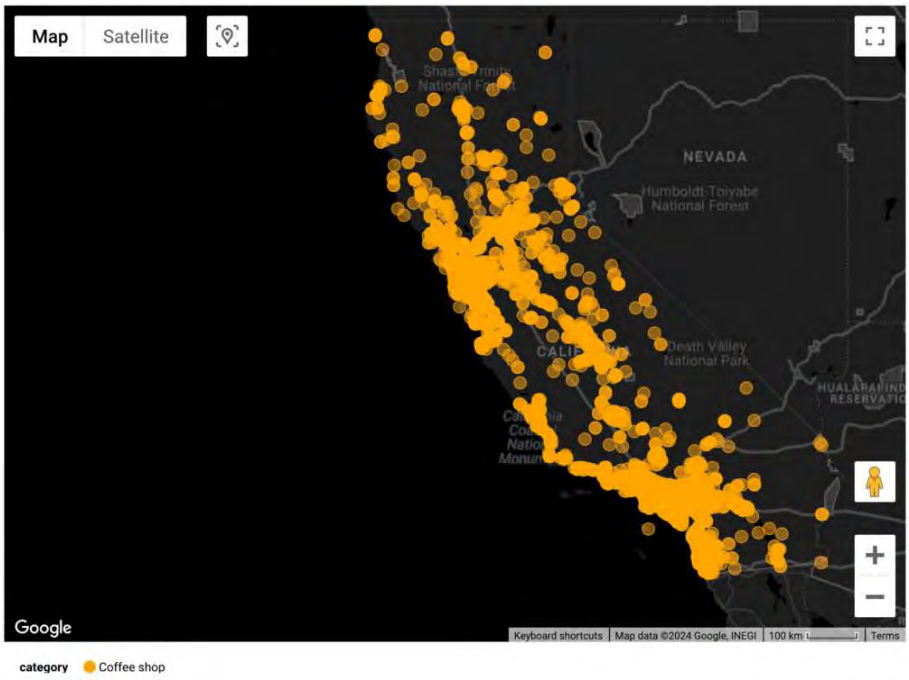
Pizza Restaurants

7,618 Coffee Shops

California has 7,618 coffee shops across the state with popular chains including Starbucks, Peet's Coffee, Dunkin and others.



1,837 Hamburger Restaurants

California also boasts a significant number of hamburger resta

Hamburger Restaurants

Conclusion

California's restaurant scene is one of the largest in the United States and offers an enormous variety of restaurants to food lovers across the state. From Mexican cuisine to fast food, there is something for everyone in California and finding a restaurant shouldn't be a problem with an average of almost two restaurants per square mile in the entire state.

Get The Latest Restaurant Trends, Guides & Tips

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FAQ

- **Which city in California has the best restaurants?**
- **How many 3 star restaurants are in C**
- **What food is LA known for?**

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

February 10, 2023

View Data Release

An important component of assessing the hazards of anticoagulant rodenticides to non-target wildlife are observations in exposed free-ranging individuals. The objective of this study was to determine if environmentally realistic, sublethal first-generation anticoagulant rodenticide exposures via prey, can result in direct or indirect adverse effects to free-flying raptors. We offered black-tailed prairie dogs (Cynomys ludovicianus) that had fed on Rozol® Prairie Dog Bait (0.005% active ingredient chlorophacinone) to six wild-caught red-tailed hawks (RTHA, Buteo jamaicensis), and uncontaminated black-tailed prairie dogs to two wild-caught RTHAs for 7 days. On day 6, blood was collected from all eight RTHAs to determine chlorophacinone's effects on blood clotting time. Russell's viper venom time and prothrombin time of chlorophacinone-exposed RTHAs exceeded mean values of reference RTHAs by more than 5 standard deviations. The observed coagulopathy confirmed that the RTHAs in the treated group were exposed to and adversely affected by chlorophacinone via secondary exposure through diet. On day 7, the RTHAs were fitted with tail-mounted VHF radio telemetry transmitters. Red-tailed hawks were release eir fate

(survival, activity, and overt physiological condition) was monitored for 33 days. Four of these six chlorophacinone-exposed free-flying RTHAs exhibited ptiloerection, an indication of thermoregulatory dysfunction due to chlorophacinone toxicity, but no signs of intoxication were observed in the reference hawk. It is noteworthy that prothrombin time values were associated with the duration and frequency of ptiloerection. These findings demonstrate that sublethal chlorophacinone exposure can directly or indirectly evoke adverse effects in wild birds. Furthermore, our anecdotal observations suggest that duration and frequency of ptiloerection from low level CPN exposures may be modulated by territoriality and mate presence. Although our sample sizes were small, this study is a first to relate coagulation times to adverse clinical signs in free-ranging birds.

Citation Information

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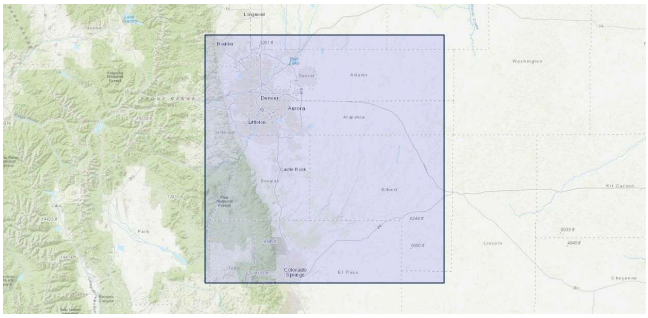
Study Area



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A Comprehensive Guide for Grocery Store in California 2025

By **Youssef Shehab**

May 21, 2024 12 mins read

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Table Of Content

California's grocery sector is a dynamic marketplace integral to the state's economy, facilitating the flow of goods from agricultural producers to a varied consumer base.

> *"Food for the body is not enough. There must be food for the soul." — Dorothy Day*

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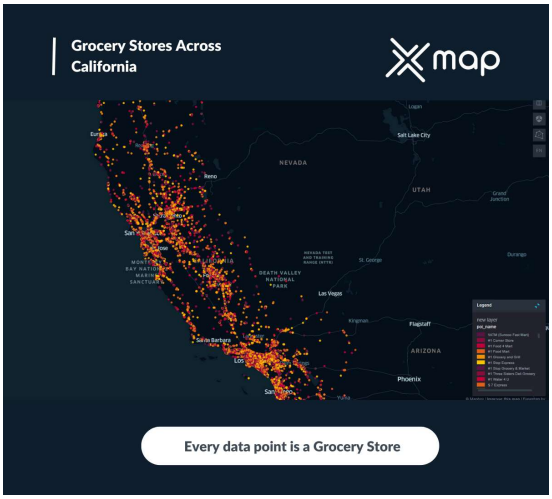
Reflecting on Dorothy Day's words, California's grocery stores do more than just feed the body; they cater to the soul by connecting people with quality food from diverse cultures and origins.

How Many Grocery Stores are in California?

California boasts a vast network of 30,132 grocery stores, offering an extensive range of options from large supermarkets to niche specialty stores. This vast array ensures accessibility to diverse food options contributing to the diverse grocery stores landscape in the USA and the state's eclectic tastes and dietary needs.



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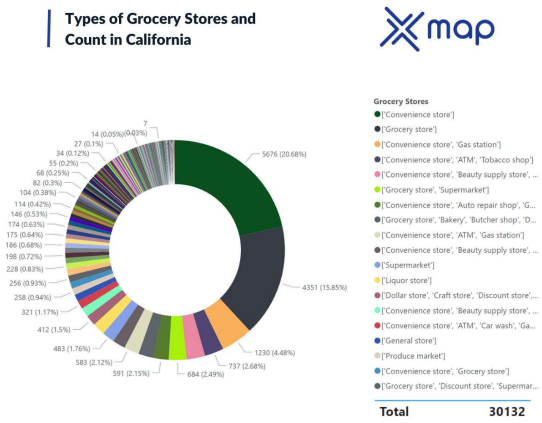
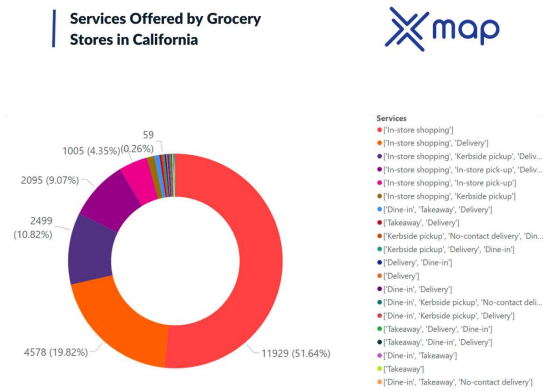


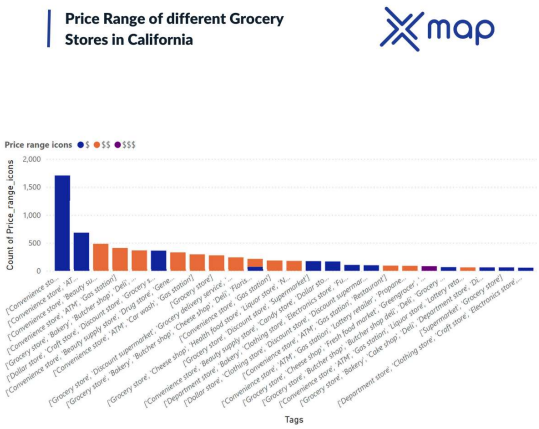
Services Offered by Grocery Stores in California

The grocery stores across California have adapted to modern consumer demands by offering various services beyond traditional shopping. A significant portion provides in-store shopping combined with delivery and pickup options, reflecting a shift towards more flexible and convenient shopping experiences.



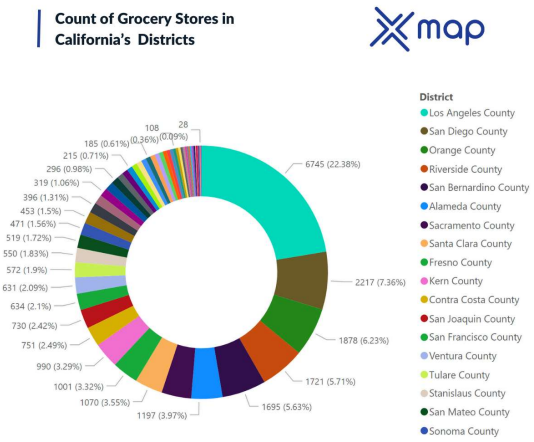
Price Range of Different Grocery Stores in California

The pricing within California's grocery sector varies widely. Stores range from budget-friendly supermarkets to high-end specialty outlets offering gourmet and organic products, ensuring that diverse consumer demographics across the state can access shopping experiences aligned with their income levels and lifestyle preferences.



Distribution of Grocery Stores Across California's Districts

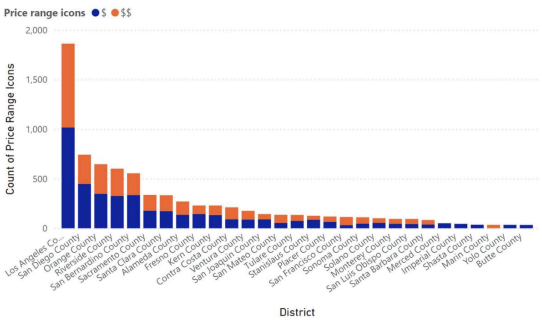
With a strategic spread across the state, the highest concentration of grocery stores is found in populous counties like Los Angeles and San Diego, ensuring optimal accessibility for all residents, regardless of their location.



Price Range of Grocery Stores Across California's Districts

The price range varies notably across different districts. In wealthier neighborhoods, consumers often find premium grocery stores and luxury food markets, while in less affluent areas, retailers offer affordable grocery options to cater to budget-conscious shoppers. This localized pricing strategy helps businesses effectively align with community-specific economic profiles.

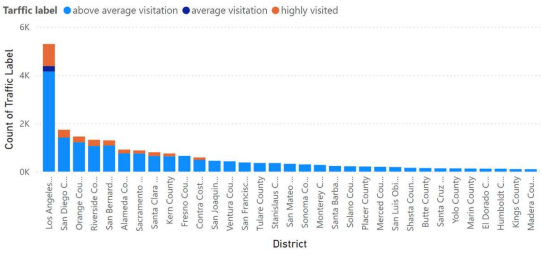
Price Range of Grocery Stores in California's Districts



Most Visited Grocery Stores in California's Districts

Los Angeles County leads with the highest foot traffic in grocery stores, making it a critical area for grocery businesses due to its vast consumer base and high demand.

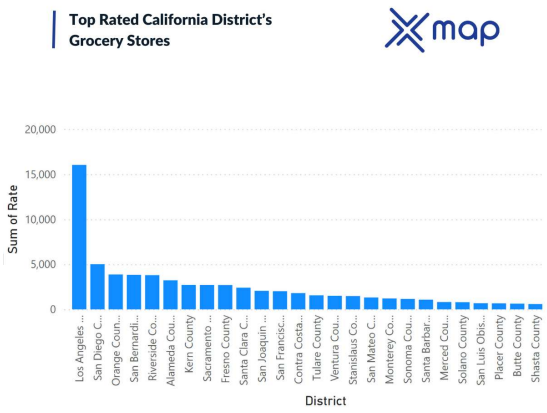
Most Visted California District's Grocery Stores



Top Rated Grocery Stores

in California's Districts

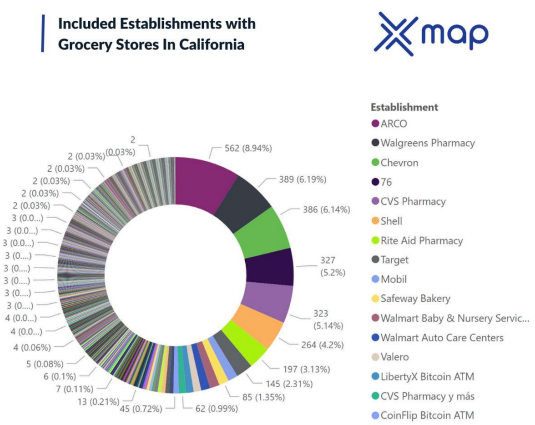
Stores in Los Angeles County are not only the most frequented but also rank highest in customer satisfaction, emphasizing the importance of quality and customer service in retaining and attracting consumers.



Diverse Establishments Featuring Grocery Stores in California

California's market is notable for its variety of establishments incorporating grocery stores, such as major pharmacies and gas stations, offering groceries alongside their primary goods. This trend highlights the evolving nature of retail,

blending different services to provide comprehensive solutions to consumer needs.



How xMap Grocery Store Data Can Help Businesses

xMap provides invaluable data on California's grocery stores, aiding businesses in refining their operational strategies or entering new market segments. Here's how xMap can support your business decisions:

Strategic Site Selection: Use demographic and competitor analysis data to identify the best locations for new outlets.

Price Optimization: Adjust pricing strategies based on thorough local

market data and competitor pricing to ensure competitiveness.

Enhancing Customer Experience: Leverage customer feedback and service trend data to enhance satisfaction and loyalty.

Market Expansion: Pinpoint underserved areas for potential expansion, utilizing targeted marketing strategies.

Conclusion

xMap's robust insights into the grocery store sector equip businesses with the necessary tools to navigate and succeed in California's competitive market. For personalized strategies and deeper insights, reach out to xMap at sales@xmap.ai. Utilize the power of xMap to drive your business forward in California, where data-driven decisions and in-depth local understanding pave the way for success in the grocery industry. Businesses exploring international retail strategies can also benefit from insights like those found in Sazka stores in the Czech Republic, which highlight how

localized data can guide smart decision-making.

Get in Touch

Whatever your goal or project size, we will handle it.
We will ensure you 100% satisfication.

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> *"We focus on delivering quality data tailored to businesses needs from all around the world. Whether you are a restaurant, a hotel, or even a gym, you can empower your operations' decisions with geo-data."*

Mo Batran
CEO & Founder @ xMap

Name Phone

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How can xMap support your project's goals? Share with us more!

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