

FINAL REPORT

for

Vertebrate Pest Control Research Advisory Committee

STUDY TITLE:

An assessment of secondary impacts of anticoagulant rodenticides on predators

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ABSTRACT

Rodents cause extensive damage to agriculture and water storage structures, and they pose a serious human health risk in many settings, as well. The use of anticoagulant rodenticides (ARs) is one tool frequently incorporated into an Integrated Pest Management approach, yet this management strategy is under substantial scrutiny given potential secondary toxicity risks to nontarget predators and scavengers. That said, many of the studies currently available for assessing this potential risk are based on biased data sets (e.g., animals taken to rehabilitation centers or road-killed animals). Therefore, we initiated a study to assess residual anticoagulant residues in liver tissues of coyotes collected mostly from rural areas of California to provide a less biased assessment of their exposure in such areas. For this study, we used coyote livers (172 samples across 24 counties) that were obtained from depredation and hunter-killed animals. The majority of samples were collected from rural sites ($n = 119$), although we opportunistically collected samples in urban ($n = 9$) and rural-urban interface areas ($n = 14$), as well. We were unable to pinpoint locations along the rural-urban gradient for 30 samples. In general, we observed greater exposure and residual concentrations of ARs in males than in females. A regional assessment showed that anticoagulant exposure was greatest in the southern desert and Central Valley regions and less in coastal and mountainous regions. Residual concentrations were far greater for second-generation anticoagulant rodenticides (SGARs) in the southern region than for SGARs in any other region or for first-generation anticoagulant rodenticides (FGARs) in any region. Both exposure rates (FGARs: rural = 26%, urban = 78%; SGARs: rural = 30%, urban = 100%) and residual concentrations (FGARs: rural = 36 ppb, urban = 129 ppb; SGARs: rural = 39 ppb, urban = 789 ppb) of ARs were far greater in urban areas than in rural sites, suggesting that agricultural uses of ARs have less impact on coyotes and potentially other nontarget predators and scavengers. This study provides a less biased data set on AR secondary exposure risk to nontarget wildlife and should assist AR regulatory reviews.

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INTRODUCTION

There are few pests that have as broad of an impact on humans as rodents, nor do many pose a greater financial burden (e.g., national estimate of damage from rats [*Rattus* spp.] alone estimated at \$27 billion annually; Pimentel 2007). Forms of rodent damage vary but include extensive damage to a wide variety of crops, forests, and rangelands (Howard et al. 1959, Capp 1976, Shwiff et al. 2009). Rodents cause substantial damage to water storage structures (Bayoumi and Meguid 2011) and irrigation systems (Montazar et al. 2017). They also pose a health and safety hazard to livestock and humans (Centers for Disease Control and Prevention 2012, Kilonzo et al. 2013), clearly showing that management of rodents is essential.

Current strategies for managing rodents generally rely on an Integrated Pest Management approach that incorporates the use of multiple techniques including habitat modification, cultural practices (e.g., deep tillage, flood irrigation), exclusion, trapping, burrow fumigation, and rodenticides (Engeman and Witmer 2000, Baldwin et al. 2014). Of these approaches, rodenticides are often the preferred tool given that they are highly efficacious and relatively quick and inexpensive to apply (Baldwin et al. 2014). The most commonly used rodenticides in California, and likely the entire western U.S., are anticoagulants (Timm et al. 2004), due in part to availability of an antidote (Vitamin K) that is lacking for most other rodenticides.

Anticoagulants are divided into two categories: first and second generation. First-generation anticoagulants (FGARs; warfarin, chlorophacinone, and diphacinone) are called so because they were developed first; they generally require multiple feedings to obtain a lethal dose (Rattner et al. 2014). It is this multiple-feed requirement that has led to their frequent use for rodent control in agricultural areas since nontarget species that feed on the product a single time usually will not receive a lethal dose. However, rodents can develop a resistance to FGARs after repeated exposure over many years (Rattner et al. 2014). This led to the development of second-generation anticoagulants (SGARs; brodifacoum, bromadiolone, difethialone, and difenacoum) which will kill the target animal after a single feeding. These products have substantially longer persistence in animals (e.g., brodifacoum > 1 year) when compared to FGARs (e.g., diphacinone \leq 40 days; Crowell et al. 2013). Given their greater potency, combined with increased longevity in animals, SGARs have rarely been approved for field use in the U.S.; the risk of secondary toxicity is considered too high (Eason et al. 2010). Their use has instead been focused on commensal rodents (Norway rats [*Rattus norvegicus*], roof rats [*Rattus rattus*], house mice [*Mus musculus*]) which cause substantial damage and health risk in urban areas and around farm buildings (Meerburg and Kijlstra 2007). Even with these restrictions, SGARs, particularly brodifacoum and bromadiolone, are by far the most common rodenticides reported in sampled animals; FGARs have typically shown up in far fewer samples (e.g., Riley et al. 2007, McMillin et al. 2008, Lima and Salmon 2010, McMillin 2012, Geduhn et al. 2015). This has led to greater restrictions on SGAR use, but also for FGARs as well.

Secondary toxicity risk from anticoagulants has been met with increasing concern by the general public in recent years, as evidenced by the proposal of California Assembly Bills 2596 (2016) and 1687 (2017) that sought to ban the use of all anticoagulants statewide, except in production agriculture areas. Much of this push to limit or ban anticoagulant use was driven by reported exposure levels in sampled wildlife species. However, these studies had substantial limitations. First off, samples frequently came from potentially biased sources that may not be representative

of their populations as a whole (e.g., animals taken to rehabilitation centers, road-killed animals, or carcasses collected from research animals; Lima and Salmon 2010, Murray 2011, Gabriel et al. 2012). Additionally, samples were often taken over a relatively small area (e.g., Riley et al. 2007, McMillin et al. 2008), and as such, may not be representative of a larger geographic area. Less biased assessments of animals collected from a large geographic area would provide a better understanding of the true rate of exposure of anticoagulant rodenticides (hereafter, ARs) in predator populations.

Furthermore, studies on anticoagulant exposure only note if, and to what level, anticoagulants are found in the animal. However, we often do not know if ARs led to mortality in the animal sampled, nor if residual anticoagulants found in live animals have an impact on their long-term behavior, survival, or reproductive capabilities (Rattner et al. 2014). Because the threshold between anticoagulant exposure and subsequent potential long-term impacts is unknown, the presence of any level of anticoagulant is often assumed detrimental to the animal. This may lead to an arbitrarily high threshold for welfare concern if anticoagulant residues have little negative impact on the animal. Conversely, anticoagulants, even if present at low levels, may have negative impacts on behavior, survival, and fitness of their host. Currently, we have very little understanding as to what impact anticoagulants have on nontarget mammalian predator populations. Therefore, we established the following initial Objectives to help provide less-biased information to better inform legislators and the general public on anticoagulant exposure levels to nontarget predators: (1) determine anticoagulant exposure and residual concentrations in coyotes (*Canis latrans*) across 4 geographical regions of CA, (2) compare anticoagulant exposure and residual concentrations in coyotes by sex and age class, and (3) explore the impact of the rural-urban gradient on anticoagulant exposure and residual concentrations in coyotes.

MATERIALS AND METHODS

Coyote liver samples were collected opportunistically through depredation events and hunter-related mortality from winter 2018 through summer 2021. All collected samples were frozen for later analysis. Collectors were asked to provide general location data, sex, and age class (juvenile [<1 year of age] vs. adult) for each sampled animal. This study was established to better identify AR residues in rural environments, and as such, most samples came from rural locations. However, some samples were obtained from urban and rural-urban interface locations, and we have included these in our study, as well. Liver samples were shipped to the Texas A&M Veterinary Medical Diagnostic Laboratory in College Station, TX, for testing. We analyzed liver tissues for the presence of warfarin, chlorophacinone, diphacinone, brodifacoum, bromadiolone, difethialone, and difenacoum using the quick, easy, cheap, effective, rugged, and safe method (QuEChERS) (Anastassiades et al. 2003). Specific details on this process can be found in Baldwin et al. (2021).

For analysis, we determined exposure as any presence of an AR. For residual concentrations, we reported values in parts per billion (ppb). We report data based on each unique AR, and we also combined ARs into FGAR (chlorophacinone, and diphacinone) and SGAR (brodifacoum, bromadiolone, and difethialone) categories. We also documented warfarin, coumatetralyl, and difenacoum. However, warfarin and coumatetralyl were documented only in one animal each, and difenacoum was only detected at trace amounts in 6 individuals. As such, they were excluded from further analysis.

For regional assessments, we placed all samples into 1 of 4 regions: (1) coastal, (2) Central Valley, (3) mountain, and (4) southern desert (Fig. 1). We also placed samples into 1 of 4 rural-urban gradient categories based on location data provided by the collector and our interpretation of this location data: (1) rural, (2) interface, (3) urban, and (4) unknown.

RESULTS

We collected 172 liver samples across 24 counties (Fig. 1). Most samples were collected in rural environments ($n = 119$), although we collected some in urban ($n = 9$) and rural-urban interface areas ($n = 14$), as well. We could not identify the collection location for some samples; these locations were marked unknown ($n = 30$). We collected the greatest number of samples in the Central Valley and mountain regions ($n = 64$ and 52 , respectively), and lowest in the southern desert and coast regions ($n = 37$ and 19 , respectively). The percentage of coyotes exposed to FGARs was greatest in the southern desert and Central Valley but was substantially lower in the mountain and coast regions (Fig. 2). SGAR exposure was much more prevalent in the southern desert, intermediate in the Central Valley and mountain regions, and lowest in the coast region. Collectively, the percentage of coyotes exposed to ARs was highest in the southern desert and Central Valley, and lowest in the coast region (Fig. 2). Residual concentrations mirrored these results except that residual levels of SGARs, and subsequently all ARs combined, were much higher for the southern desert than all other regions (Fig. 3). It is worth noting that FGAR residues were substantially lower than that observed for SGARs. The substantial prevalence of SGARs in the southern desert was largely driven by high bromadiolone residues in coyote livers (Fig. 4).

Rodenticide exposure and residual concentrations of ARs was generally greatest for males (Figs 5–7). This held true for all ARs except for brodifacoum (Fig. 7). FGAR exposure and residual concentrations were also impacted by age class, with exposure greatest for adults (Fig. 8–10). The trend was less clear for SGARs, as exposure was similar between both age classes (Fig. 8). However, residual concentrations of SGARs were a bit higher for juveniles (Fig. 9), although brodifacoum again exhibited the opposite response (Fig. 10).

Exposure and residual concentrations of ARs were greatest in the urban environment, intermediate in urban-rural interface and unknown localities, and lowest in rural sites (Figs. 11–13). In fact, residual concentrations of all ARs were many times lower in rural environments than in all other locations, indicating that AR exposure in coyotes appears to be largely driven by urban and adjacent localities (Fig. 12–13). Bromadiolone was heavily present in all areas but rural sites (Fig. 13). All other ARs except chlorophacinone were most prevalent in urban areas (Fig. 13). It bears noting that diphacinone residues were ~8 times greater in urban areas than in residential sites (Fig. 13). Collectively, 50 of the 119 coyotes sampled from rural sites exhibited some level of AR exposure (Table 1). Residual concentrations of FGARs and SGARs in rural coyotes were similar, resulting in a mean residual concentration of ARs of 81 ppb (Table 1).

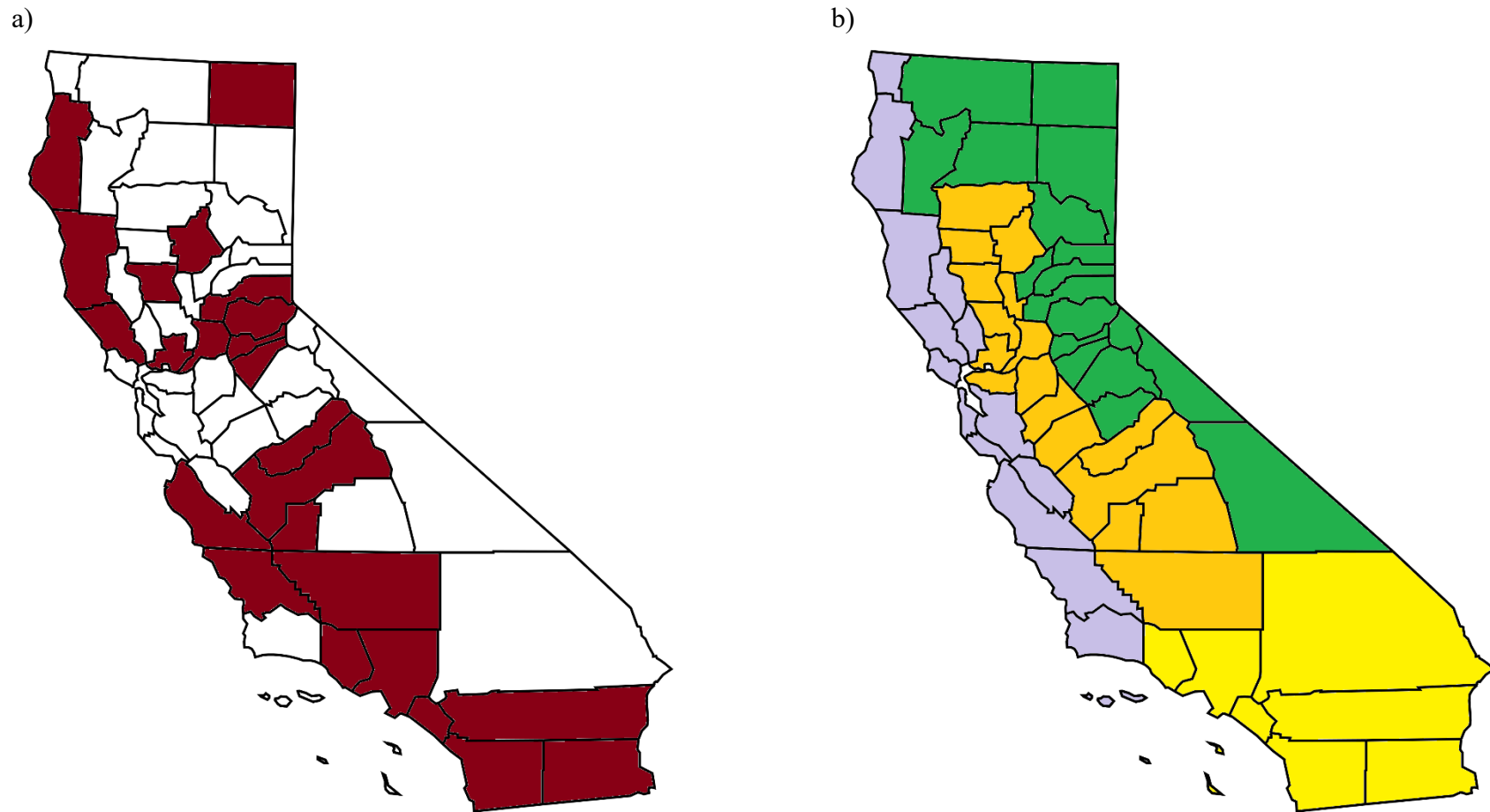


Fig. 1. Map of California indicating counties (a) where coyote liver samples were collected (red = county where at least one liver sample was collected), and a map of California counties (b) delineating the different regions where samples were collected from: purple = coast, green = mountain, orange = Central Valley, and yellow = southern desert.

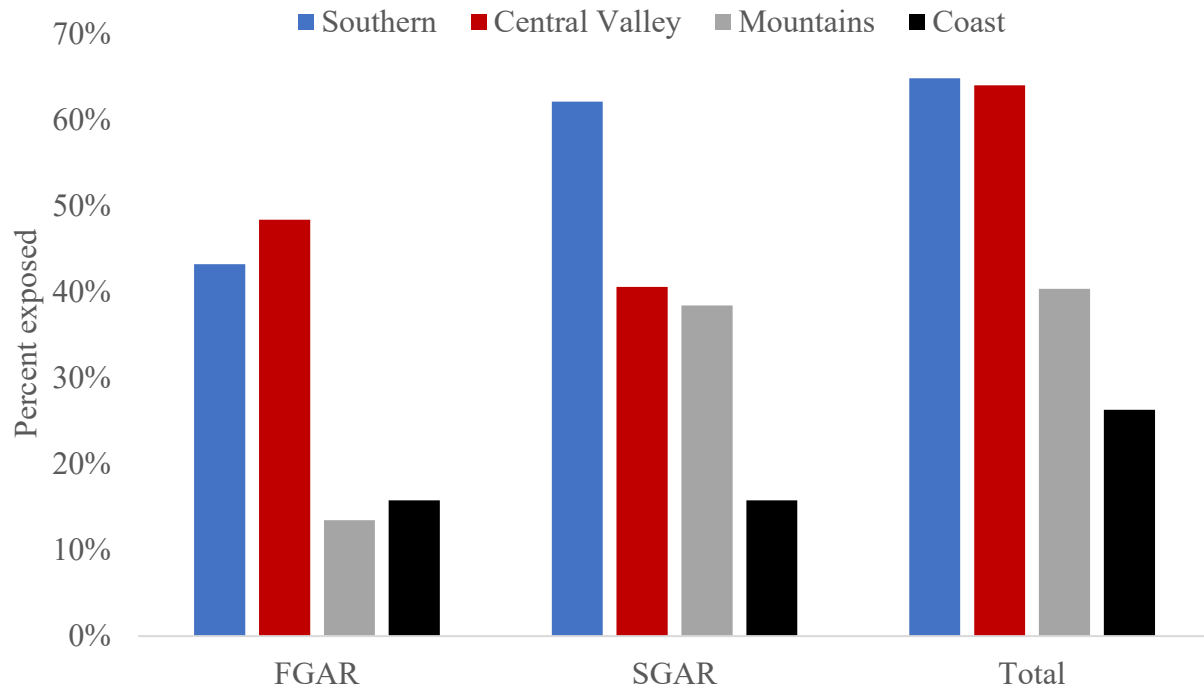


Fig. 2. The percentage of sampled coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure based on different regions that were sampled throughout California.

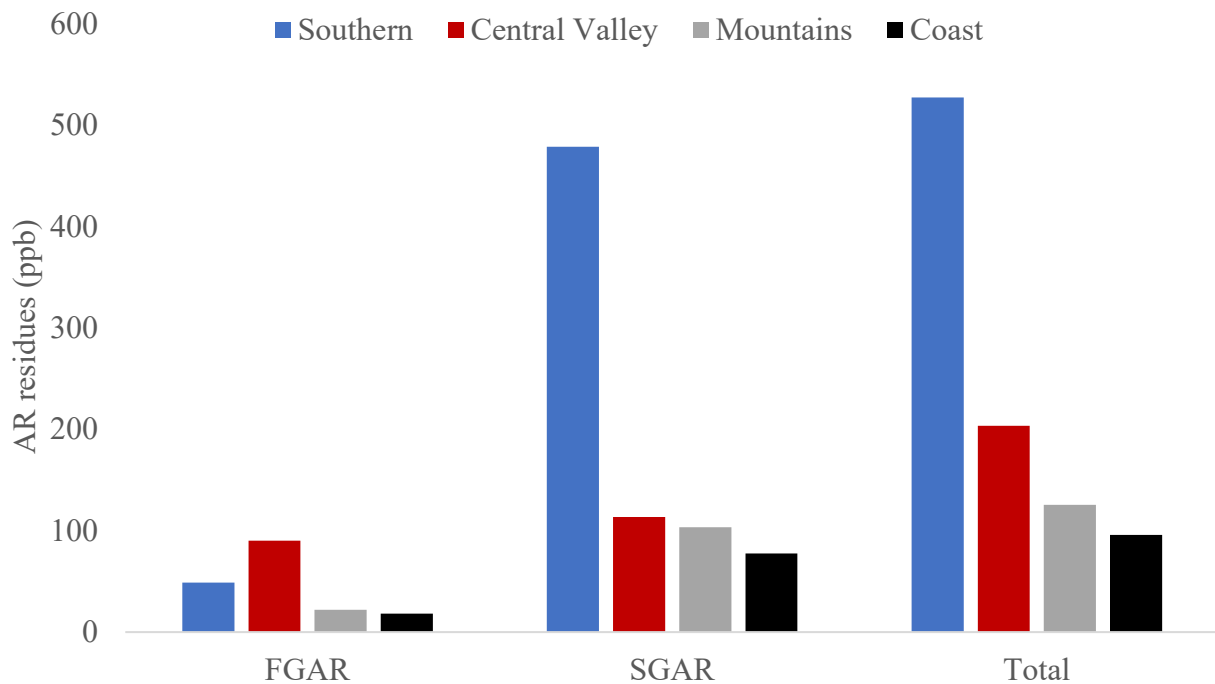


Fig. 3. Anticoagulant residues for sampled coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure based on different regions that were sampled throughout California.

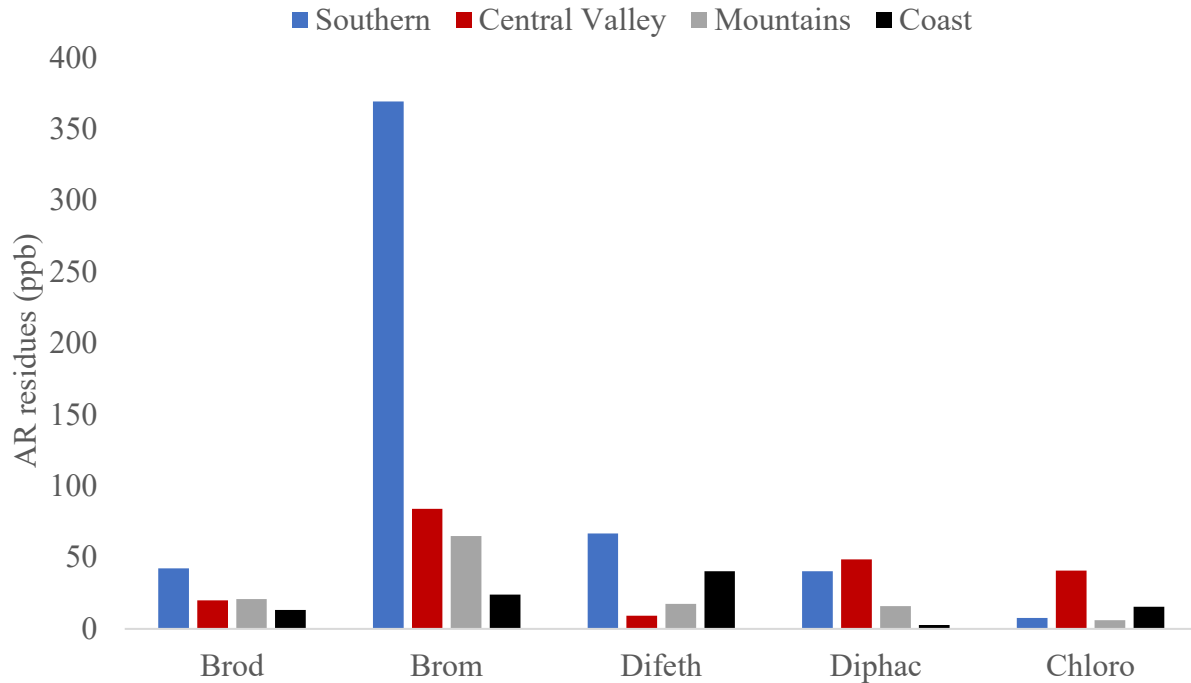


Fig. 4. Anticoagulant residues for sampled coyotes that were exposed to brodifacoum (Brod), bromadiolone (Brom), difethialone (Difeth), diphacinone (Diphac), and chlorophacinone (Chloro) based on different regions that were sampled throughout California.

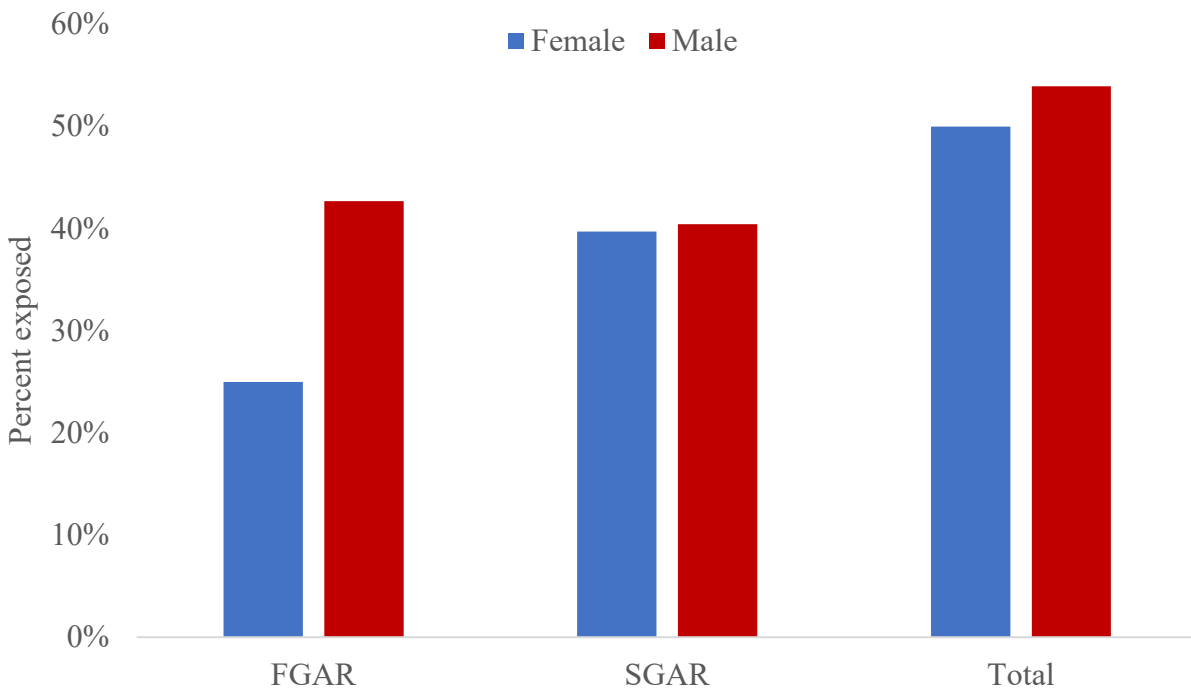


Fig. 5. The percentage of sampled female and male coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure throughout California.

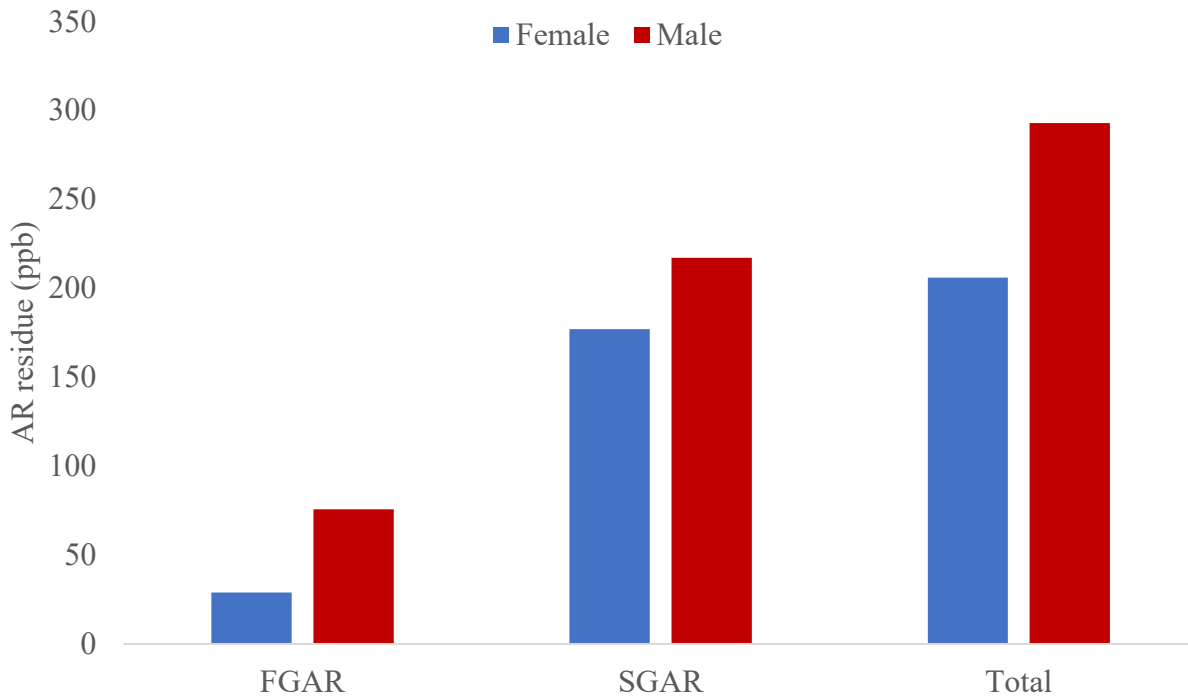


Fig. 6. Anticoagulant residues for sampled female and male coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure throughout California.

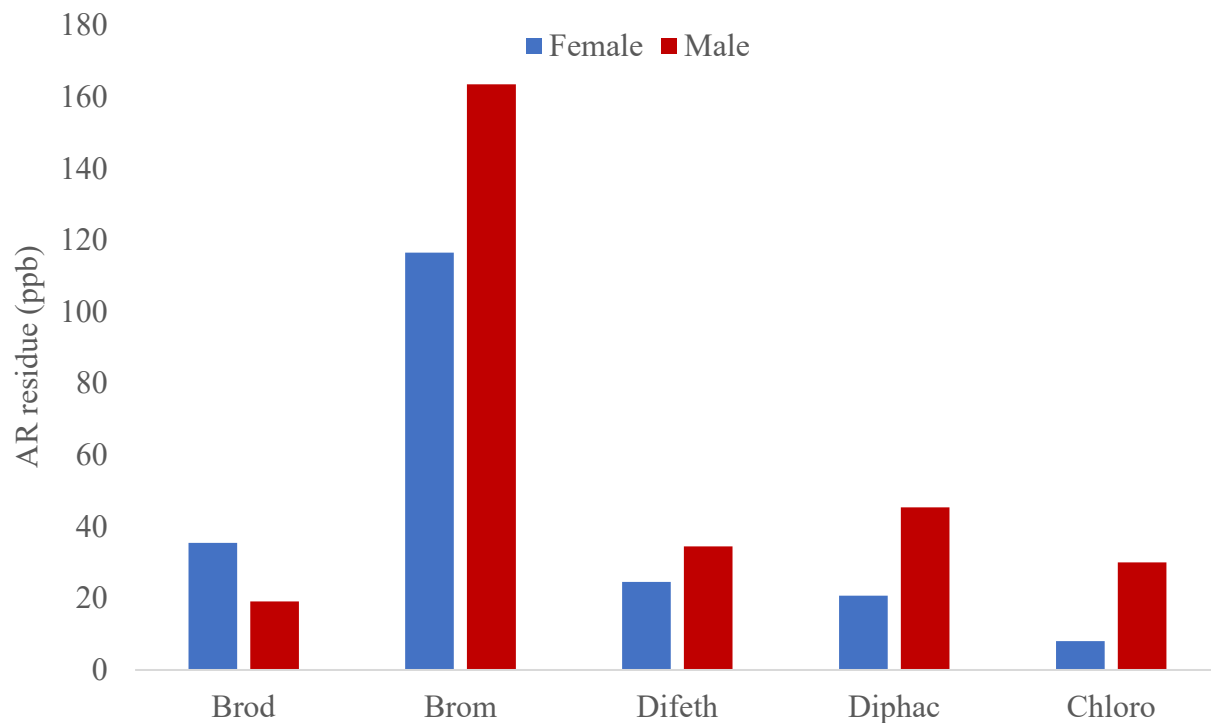


Fig. 7. Anticoagulant residues for sampled female and male coyotes that were exposed to brodifacoum (Brod), bromadiolone (Brom), difethialone (Difeth), diphacinone (Diphac), and chlorophacinone (Chloro) that were sampled throughout California.

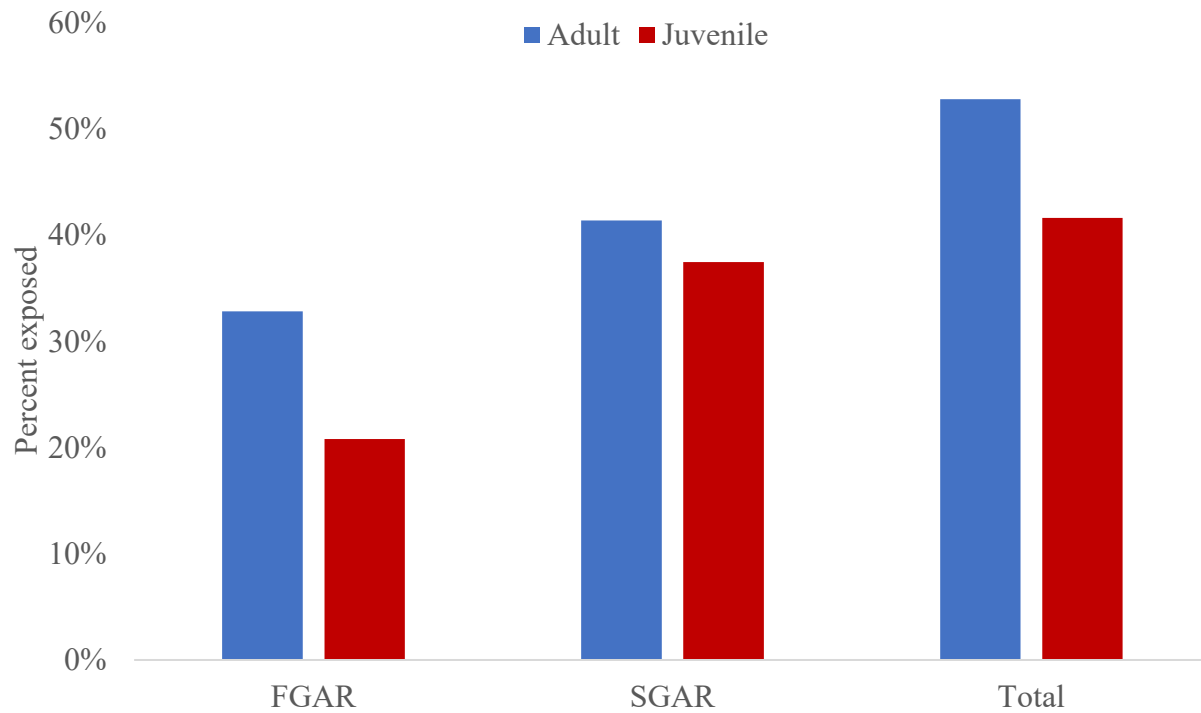


Fig. 8. The percentage of sampled adult and juvenile coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure throughout California.

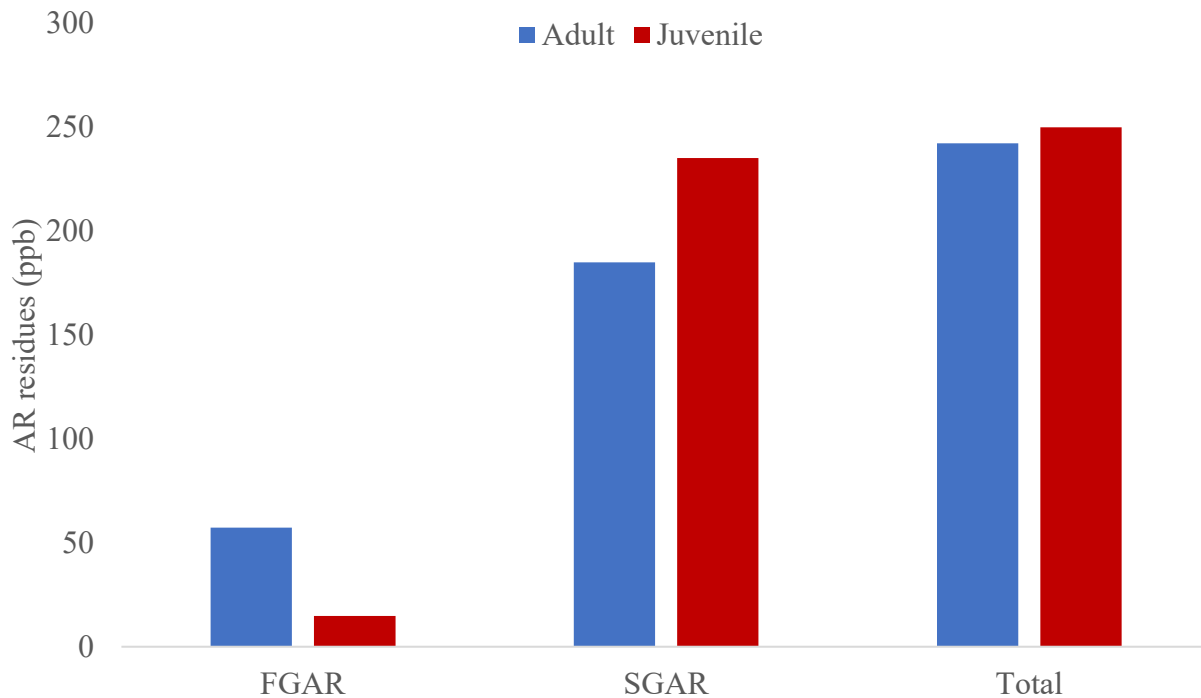


Fig. 9. Anticoagulant residues for sampled adult and juvenile coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure throughout California.

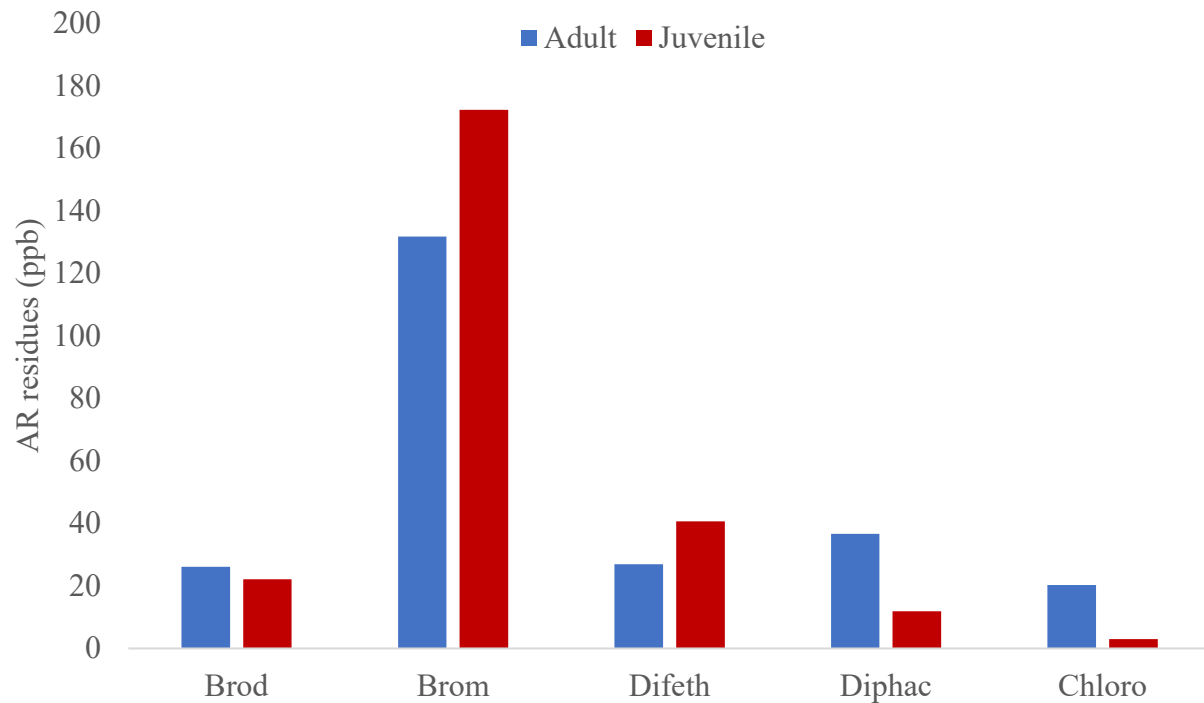


Fig. 10. Anticoagulant residues for sampled adult and juvenile coyotes that were exposed to brodifacoum (Brod), bromadiolone (Brom), difethialone (Difeth), diphacinone (Diphac), and chlorophacinone (Chloro) that were sampled throughout California.

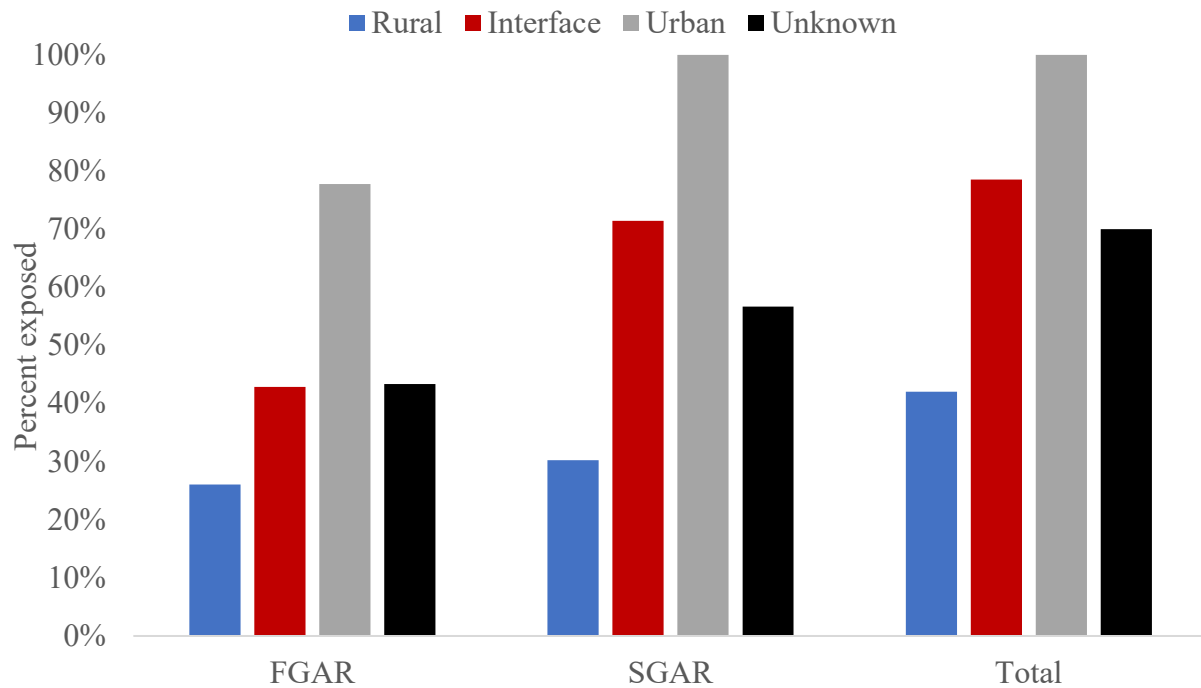


Fig. 11. The percentage of sampled coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure based on rural, urban, rural/urban interface, and unknown urbanization categories sampled throughout California.

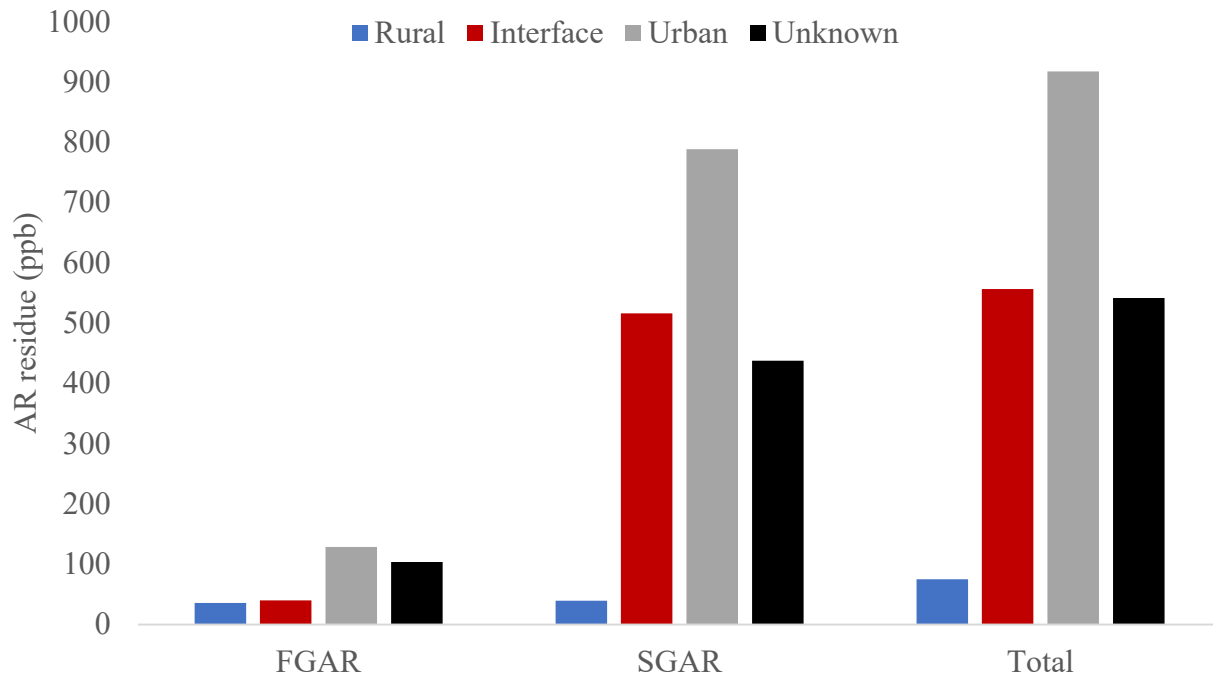


Fig. 12. Anticoagulant residues for sampled coyotes that were exposed to FGARs, SGARs, and combined FGAR and SGAR (Total) exposure based on rural, urban, rural/urban interface, and unknown urbanization categories sampled throughout California.

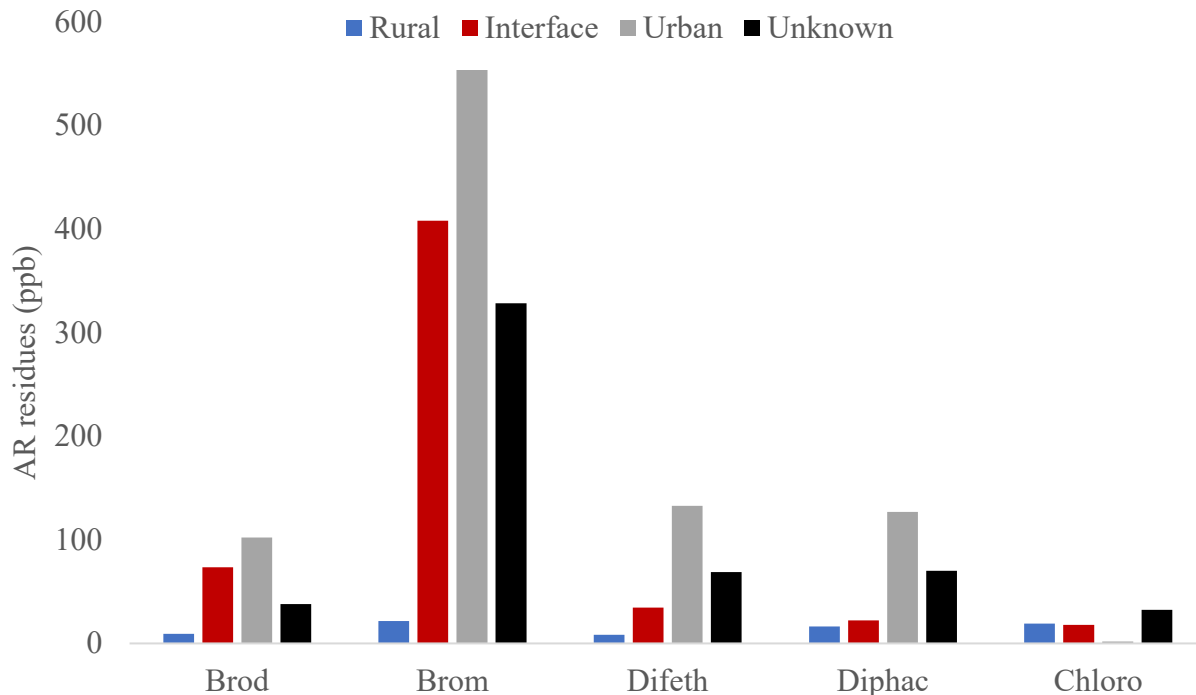


Fig. 13. Anticoagulant residues for sampled coyotes that were exposed to brodifacoum (Brod), bromadiolone (Brom), difethialone (Difeth), diphacinone (Diphac), and chlorophacinone (Chloro) based on rural, urban, rural/urban interface, and unknown urbanization categories sampled throughout California.

Table 1. The number of liver samples where an anticoagulant rodenticide was detected, the maximum parts per billion (ppb) for a liver sample, and the mean ppb for all liver samples collected from rural coyotes ($n = 119$) throughout California from 2018–2021. Anticoagulant rodenticides tested included brodifacoum (Brod), bromadiolone (Brom), difethialone (Difeth), diphacinone (Diphac), and chlorophacinone (Chloro). We also combined data for first-generation anticoagulant rodenticides (FGARs; diphacinone and chlorophacinone) and second-generation anticoagulant rodenticides (SGARs; brodifacoum, bromadiolone, and difethialone), as well as all anticoagulant rodenticides combined.

	Brod	Brom	Difeth	Diphac	Chloro	FGAR	SGAR	Combined
Number	15	29	6	28	10	31	36	50
Max ppb	613	350	758	623	1520	1528	1355	1528
Mean ppb	9	22	8	21	20	42	39	81

DISCUSSION

Numerous studies have shown substantial AR exposure of a variety of nontarget wildlife species over the last couple of decades (e.g., Riley et al. 2007, Lima and Salmon 2010, Murray 2011). However, the majority of these studies have focused on raptors, and those that have tested for AR exposure in mammalian carnivores have often utilized liver samples from road-kill animals and those brought into rehabilitation centers (e.g., Serieys et al. 2015). Our study provided a less-biased sampling approach, with a particular focus on rural localities where AR use should be more heavily skewed toward the use of FGARs for management of field rodents. As with other studies, we often detected AR exposure in sampled animals (53% of coyotes sampled). If considering only those individuals collected in rural settings, this value is somewhat lower (42% of coyotes sampled). These exposure levels are less than half that reported in urban environments (e.g., 100% and 98% of coyotes were exposed to ARs in Denver, CO and southern CA, respectively; Poessel et al. 2015, McKenzie 2021). Furthermore, residues collected exclusively from rural areas are substantially less than what has been reported in urban environments. For example, in Denver, mean residual concentrations of ARs were documented at 344 ppb (Poessel et al. 2015). In a study conducted in southern CA, mean concentrations of bromadiolone was 644 ppb by itself (McKenzie 2021), with other ARs contributing even more to total exposure (e.g., difethialone = 199 ppb, brodifacoum = 120 ppb; McKenzie 2021).

It is interesting to note that diphacinone exposure is substantially greater in urban areas (127 ppb) than in rural environments (16 ppb). Urban values in this study are similar to those reported by McKenzie (2021) in southern CA (110 ppb). This bears noting as the passage of Assembly Bill 1788 that has placed a moratorium on SGARs in most areas may lead to increased usage of diphacinone and other FGARs to combat commensal rodents. If so, we may see even greater residual concentrations of diphacinone in nontarget scavengers and predators in urban environments moving forward. Regardless, based on residual concentrations and exposure frequency of diphacinone in coyotes from rural and urban areas, it appears that most nontarget impacts from this FGAR are occurring in urban/suburban localities. As such, any future restrictions on its usage would likely have a far greater impact if they were targeted toward commensal rodent applications rather than for field rodents.

It is not surprising that the greatest exposure of coyotes to SGARs occurred in the southern desert given the heavy urbanization of this area. Bromadiolone is the most used SGAR in California (Quinn et al. 2019), and it was the primary AR that showed up in tested coyotes. There has been a concerted effort to move away from usage of brodifacoum in recent years given the high toxicity and long half-life of this product (Eason et al. 2020). This reduction in usage was apparent based on the lower residues observed for this SGAR when compared to bromadiolone.

For FGARs, we observed the greatest exposure in coyotes in the Central Valley. FGARs are commonly used rodenticides for managing field rodents in agricultural settings (Baldwin and Salmon 2011); the Central Valley is one of the largest agricultural production areas in the world, with frequent rodent damage experienced in perennial cropping systems. The greater FGAR exposure in this region may potentially be a reflection of its greater usage to combat damaging rodents in agricultural fields.

The areas with the least exposure were the mountain and coastal regions. It is not surprising that the mountain region had low exposure. These areas consist of large expanses of sparsely populated habitats. Therefore, less AR usage was anticipated in this region. In contrast, the coastal region is more densely populated. However, coastal areas are often considered more sensitive to environmental concerns than some other areas in California (Baldwin et al. 2013), and as such, less AR usage may have occurred in this area. Further investigation would be helpful to determine if AR usage was in fact lower in these regions and if this was a driving factor in the lower exposure observed in mountain and coastal counties.

Male coyotes generally exhibited greater AR exposure than females. In some California regions, male coyotes have larger home ranges than females (Riley et al. 2002), which could increase the chance that they will encounter and consume intoxicated rodents. The impact of age class on exposure was less clear. Adult coyotes exhibited greater exposure rates and residual concentrations of FGARs. However, we observed mixed results with SGARs, as exposure rate was greater for adults, but residual concentrations were greater for juveniles. McKenzie et al. (2022) also observed that adults were exposed to more ARs and had higher residual concentrations when compared to juveniles. Greater concentration of ARs in adults may simply be a reflection of longer lifespans that would allow for greater accumulation over time. However, this would not explain the greater concentration of SGARs in juveniles. Ultimately, the exact relationship between age class and AR exposure is unknown and in need of further investigation.

CONCLUSIONS

Our study focused on depredation and hunter-killed coyotes in largely rural areas throughout California, making it unique compared to most other AR exposure studies. In rural areas, AR exposure still occurs, although exposure levels appear to be substantially less than that observed in urban regions. Research must continue to identify methods to reduce AR exposure to nontarget wildlife, but at this point, it appears that the most likely avenue to reduce AR exposure is to alter how these rodenticides are used in urban environments.

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