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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.

Keywords Bobcats · Secondary poisoning · Anticoagulant rodenticides · Notoedric mange · Urbanization · Residential · Fetal transfer

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Introduction

Anticoagulant rodenticides (ARs) are toxicants increasingly recognized as a threat to nontarget wildlife (Erickson and Urban 2004; US EPA 2008; Elmeros et al. 2011; Gabriel et al. 2012; California Department of Pesticide Regulation 2013). As vitamin K antagonists, ARs interrupt the production of vitamin K-dependent blood clotting proteins, leading to the depletion of these proteins over a period of days inducing mortality by hemorrhage (Erickson

and Urban 2004). Comprised of two classes of compounds, ARs are the primary chemical method used worldwide for the control of rats and mice (Stone et al. 1999; Eason et al. 2002). First-generation ARs, including warfarin, diphacinone, and chlorophacinone, are more readily metabolized, have a shorter half-life in hepatic tissue (2 weeks to several months) (Eason et al. 2002), and must be consumed in multiple feedings to reach a lethal dose (Erickson and Urban 2004). Second-generation ARs include brodifacoum, bromadiolone, and difethialone, and were developed to target rodents with genetic resistance to warfarin (Hadler and Buckle 1992). Second-generation ARs have prolonged action and increased potency (Petterino and Paolo 2001), with hepatic half-lives ranging 6–12 months, and may persist in liver tissue for more than a year in some species (Eason et al. 2002). Both classes of compounds have delayed onset of action, and death from AR consumption can occur up to 10 days after ingestion (Cox and Smith 1992). Individual rodents may continue to accumulate the compounds over a period of days, increasing their attractiveness to predators as they become weakened by the toxicant, and are easier to capture (Cox and Smith 1992; Berny et al. 1997; Berny 2007). For predators that consume prey targeted with ARs, both acute and chronic secondary exposure to the toxicants can occur (Erickson and Urban 2004; Riley et al. 2007; Elmeros et al. 2011; Gabriel et al. 2012).

Exposure of nontarget wildlife to ARs has been documented for numerous predatory mammal and bird species (McDonald et al. 1998; Stone et al. 1999; Riley et al. 2003, 2007; McMillin et al. 2008; Walker et al. 2008; Elmeros et al. 2011). Detection rates for ARs can exceed 80–90 % in wildlife and are directly responsible for mortalities in many species including coyotes (*Canis latrans*, Riley et al. 2003), San Joaquin kit foxes (*Vulpes macrotis mutica,* McMillin et al. 2008), California fishers (*Martes pennanti,* Gabriel et al. 2012), mountain lions (*Puma concolor*; Riley et al. 2007), red kites (*Milvus milvus,* Berny and Gaillet 2008), barn owls (*Tyto alba*), barred owls (*Strix varia*) and great horned owls (*Bubo virginianus*) (Stone et al. 2003; Albert et al. 2009). Factors that lead to secondary exposure of nontarget species are complex (Eason et al. 2002; Shore et al. 2006) because exposure is related to the persistence of compounds, levels of usage, how and where the compounds are applied, and trophic ecology (Eason et al. 2002; Shore 2003; Erickson and Urban 2004; Shore et al. 2006). The accurate assessment of AR exposure in wildlife is difficult because studies often rely on post-mortem sampling of liver tissue from carcasses found opportunistically. This may lead to a bias towards detection of those compounds with the longest persistence in hepatic tissue and at lethal dosages, and an underestimation of the number of animals that are exposed to ARs.

In southern California, more than a decade of research by U.S. National Park Service biologists in and around Santa Monica Mountains National Recreation Area (SMMNRA), a national park bordering Los Angeles, has documented widespread AR exposure in multiple carnivore species. AR exposure was the second leading cause of mortality during a 9-year coyote study in which 83 % of individuals tested were exposed (Riley et al. 2003; Gehrt and Riley 2010). Approximately 90 % of mountain lions and bobcats (*Lynx rufus*) in the study area were also exposed (Riley et al. 2007; Beier et al. 2010). Using telemetry data on bobcats and mountain lions, AR toxicant load, or the concentration of AR residues detected, was positively associated with use of developed areas (Riley et al. 2007, 2010; Beier et al. 2010) suggesting that developed areas are a major source of AR contamination.

Although high rates of exposure were documented for bobcats in SMMNRA, death as a result of AR exposure was reported only once (Riley et al. 2010). However, secondary AR exposure at $\geq$0.05 ppm was significantly associated with death due to severe notoedric mange (*Notoedres cati*), an ectoparasitic disease (Riley et al. 2007). Further, a precipitous population decline and genetic bottleneck in bobcats occurred as a result of the mange outbreak from 2002 to 2006 (Riley et al. 2007; Serieys et al. 2014). Notoedric mange was previously reported only in isolated cases in free-ranging felids (Pence et al. 1982; Maehr et al. 1995; Pence et al. 1995), however, the disease may be increasing in bobcats across California (Serieys et al. 2013; Stephenson et al. 2013). To date, all bobcats with mange that have been tested were positive for ARs ($N = 19$, Riley et al. 2007; $N = 11$, Serieys et al. 2013). These correlative findings suggest that chronic, sublethal exposure to ARs may influence immune function in bobcats, increasing their susceptibility to mange infestation and decreasing anti-mite immune response (Riley et al. 2007; Serieys et al. 2013).

We investigated risk factors for exposure to ARs in bobcats from two areas in southern California: in the SMMNRA area northwest of Los Angeles, and in Orange County to the southeast. We used blood and liver to detect exposure to ARs across varied landscapes that included fragmented urban and protected natural areas. Liver samples were collected postmortem to evaluate exposure history of individuals. Blood samples were collected primarily during animal capture to evaluate recent exposure. We used multiple measures of AR exposure including prevalence of exposure to any AR, prevalence of exposure to specific ARs, the number of different compounds detected, and compound residue concentrations (toxicant load). We evaluated AR exposure from 1997 to 2012 as part of the long-term study at SMMNRA, and from 2006 to 2010 in Orange County. We assessed risk factors for exposure

including sex, age, season, and landscape characteristics, specifically proximity to residential, commercial, and other developed areas. Using a much larger number of samples collected over a longer period of time and from a greater geographic area than a previous study (Riley et al. 2007), we examined the potential association between ARs and notoedric mange by evaluating the association between mange and a range of residue concentrations and the number of compounds detected.

Methods

Study area and sample collection

Sampling primarily occurred in two areas (Fig. 1). In Los Angeles and Ventura Counties, samples were collected by NPS and University of California, Los Angeles (UCLA) biologists from 1997 to 2012 during an ongoing NPS bobcat ecology study in SMMNRA (Riley et al. 2003, 2006, 2007, 2010; Serieys et al. 2013; Serieys et al. 2014). The eastern boundary of SMMNRA is less than 10 km from downtown Los Angeles and the park encompasses both large regions of continuous protected habitat with minimal urban development, including state and national park lands, and highly fragmented areas with intense urban development. In the Orange County study area (OCSA), bobcats were sampled from 2006 to 2010 by the U.S. Geological Survey (USGS) across a network of public nature reserves within landscapes experiencing rapid urbanization and near the more protected Santa Ana Mountains (Lyren et al. 2006, 2008; Poessel et al. 2014). The Santa Ana Mountains straddle Riverside, Orange, and San Diego Counties but most of the samples (93 %) were collected in Orange County. Anthropogenic development across both study areas includes residential, commercial, and agricultural development, as wells as many "altered open" areas such as golf courses and landscaped parks (Table 1). Samples were also opportunistically collected in two additional areas north and south of our study areas in San Barbara ($N = 3$) and San Diego Counties ($N = 8$) when animals died in wildlife rehabilitation facilities or were reported dead by residents.

Bobcats were captured and handled as previously described (Riley et al. 2003, 2006, 2007; Serieys et al. 2013) with approval by the Office of Animal Research Oversight of UCLA (Protocol ARC#2007-167-12) and by the Colorado State University Animal Care and Use Committee (Protocol #11-2453A). Scientific collecting permits were authorized by the California Department of Fish and Wildlife (SC-9791). From 2000 to 2009, the majority of trapping efforts occurred from mid-October to mid-February, and thus collected during the non-breeding, wet season (November 1–April 30). Individuals were chemically immobilized with a mixture of ketamine HCl (10 mg/kg) and xylazine HCl (1 mg/kg) or ketamine HCl (5 mg/kg) and medetomidine HCl (0.1 mg/kg). We recorded age class, sex, weight, and morphological measurements (i.e., chest circumference, body length, tail length, ear length, head circumference, etc.). Individuals were classified as juveniles (<2 years) or adults (>2 years) based on body size, weight, tooth wear and eruption, and reproductive status (Riley et al. 2003, 2006). A subset of individuals were also radio-collared as part of the NPS and USGS studies (Riley et al. 2003, 2006, 2007; Poessel et al. 2014). To obtain serum samples, blood was centrifuged within 24 h of collection and serum was collected. All samples, including liver (see below), were transported from the site of collection to storage facilities on ice packs.

In both study areas, we obtained liver samples during necropsies from opportunistically found carcasses (e.g. road-kill) or from animals that died in rehabilitation centers (Table 2). In SMMNRA, when possible liver samples were also collected from radio-collared animals that died. For 20 individuals, blood and liver were simultaneously obtained postmortem (Table 2). The cause of mortality, collection date, sex, age class, and location found were recorded. All animals were visually inspected for clinical signs of notoedric mange that included severe dermatitis, alopecia, and lichenification of the skin. If clinical mange was observed, skin scrapings in the affected areas were performed to identify mite species as previously described (Riley et al. 2007; Serieys et al. 2013; Stephenson et al. 2013). To measure specific age, an upper canine tooth was extracted during necropsy to determine age in years based on cementum annuli (Matson's Laboratory LLC, Missoula, MT) (Crowe 1972). Capture and mortality locations were recorded using GPS devices. Blood, serum and liver were stored at −20 or −80 °C until tested. Anticoagulant rodenticide compounds are stable (Waddell et al. 2013) and so the length of time under refrigeration should not have affected the results.

Anticoagulant assessment

We assessed the presence and amount of warfarin, coumachlor, bromadiolone, brodifacoum, diphacinone, chlorophacinone, and difethialone in 2 g of liver tissue, 1 g of serum, or 2 g of whole blood by high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS/MS) (Riley et al. 2007; Ruder et al. 2011; Waddell et al. 2013). Samples were first screened for the presence of each AR by LC–MS/MS. Positive AR samples were then quantitated by HPLC using either UV diode array detection (diphacinone, chlorophacinone and difethialone) or fluorescence detection (warfarin, coumachlor, bromadiolone, and

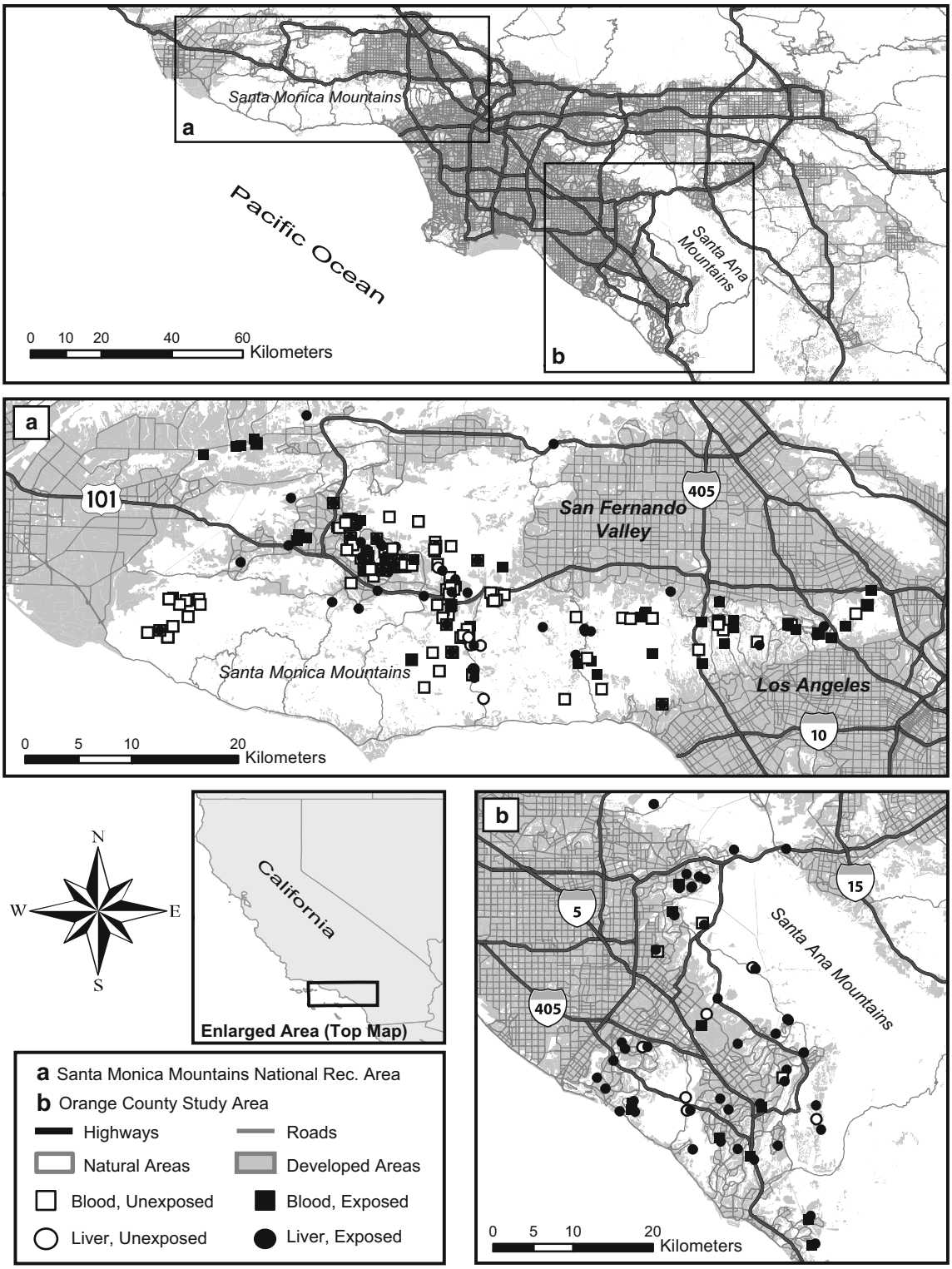

Fig. 1 Map of the study areas. **a** Santa Monica Mountains National Recreation Area (SMMNRA) and **b** Orange County Study Area (OCSA). Sampling locations and exposure results are shown. Blood sampling locations are represented with *squares* while liver sampling locations are represented with *circles*

brodifacoum). Limits of quantitation for these anticoagulants in liver tissue were 0.01 ppm for brodifacoum, 0.05 ppm for bromadiolone, warfarin, and coumachlor, and 0.25 ppm for chlorophacinone, diphacinone, and difethialone. Thirty-nine of 172 liver results were from Riley et al. (2007) (Table 2) and here we performed anticoagulant assessments using the same

Table 1 Classification of predictor land use variables used for analysis of dependent AR exposure measures

Broad classification	Specific land use tested in models	Percent of study areas			Percent of buffer zones		
		SMMNRA	OCSA	Mean	SMMNRA	OCSA	Mean
Agriculture	Crops, pastures orchards and vineyards	3.39	3.00	3.20	2.18	1.62	2.07
	Horse ranches	0.53	0.23	0.38	0.53	0.58	0.54
	Other agriculture	0.50	0.89	0.70	1.33	1.56	1.38
	Total agriculture	4.42	4.12	4.27	4.04	3.76	3.99
Commercial and industrial	Schools and religious	1.04	1.61	1.33	0.57	2.15	0.88
	Office and retail	1.29	2.89	2.09	1.18	1.20	1.18
	Mixed commercial and industrial	1.61	5.20	3.41	1.85	3.64	2.20
	Water facilities	0.34	0.57	0.46	0.49	3.79	1.13
	Total commercial and industrial	4.28	10.27	7.28	4.09	10.78	5.39
Residential	Multifamily/commercial high-density (>25 units/ha)	1.38	4.55	2.97	2.14	4.00	2.50
	Single-family high-density (5–10 units/ha)	14.80	17.04	15.92	10.76	7.58	10.14
	Single-family low-density (<5 units/ha)	5.63	1.96	3.80	3.90	8.22	4.74
	Total residential	21.81	23.55	22.68	16.80	19.80	17.38
Altered open space	Golf courses and cemeteries	1.02	1.75	1.39	0.55	2.67	0.96
	Other recreational/altered open space	0.61	1.43	1.02	0.53	1.86	0.79
	Total altered open space	1.63	3.18	2.41	1.08	4.53	1.75
Natural	Undeveloped natural	66.82	58.82	62.82	54.08	23.32	48.01

The percentage of each land use within a single polygon drawn around all bobcat buffer zones for each study area and the mean across both study areas is shown. Additionally, the mean value of each land use type across bobcat buffer zones for each study area and across all composite bobcat buffer zones is shown. The sum of land-use variables for each study area do not equal 100 % because some land-use types (e.g. open water, roads, railroads), comprising a mean of 0.55 % of the study areas, were not included in analyses

approach. In blood, limits of quantitation were 1 ppb for each compound with method detection limits ranging from 0.28 to 0.45 ppb. ARs that were determined to be positive by LC–MS/MS, but were below the limit of quantitation by HPLC, were defined as above the limit of detection (LOD) or "above LOD."

Finally, to make comparisons between AR exposure in bobcats, and the amount of toxicants applied where bobcats were sampled, we obtained data on reported use in Los Angeles, Orange, and Ventura Counties (measured in pounds) as posted in the California Department of Pesticide Regulation online database from 1997 to 2012 (http://www.cdpr.ca.gov/docs/pur/purmain.htm) for the four most commonly detected compounds. Records for Orange County were accessed only for the years for which we had samples from the study area (2006–2010). We averaged the pounds applied across the counties for each sample year (see Fig. 2c, Supplemental Fig. S1c).

Land use analysis

To evaluate the land use characteristics of surrounding landscape for all sampled bobcats, we created circular buffer zones with each capture or mortality location as the center. Each buffer zone was equal to the area of an average home range (95 % minimum convex polygon) for animals that have been radio-tracked in each study area (males: 5.2 km^2 SMMNRA; 5.6 km^2 OCSA; females: 2.3 km^2, SMMNRA; 3.2 km^2 OCSA) (Riley et al. 2010). Animals that were sampled in Santa Barbara and San Diego Counties were excluded from land use analysis because exact sampling locations were unavailable. We used the 2005 land use dataset provided by Southern California Association of Governments (SCAG, http://gisdata.scag.ca.gov/Pages/Home.aspx) with bobcat buffer zones in ArcGIS 10.1 (ESRI, Redlands, CA) to quantify land use types for each bobcat. Seventy-six land use types were included in bobcat buffer zones. These land use types were grouped into five general classes including: (1) agriculture; (2) commercial and industrial; (3) residential; (4) altered open areas such as landscaped parks, golf courses, and cemeteries; and (5) undeveloped natural areas (Table 1). We merged the 76 SCAG land use variables into 13 groups that were broadly characterized into five classes of land uses based on similarity and relevance to this study (Table 1, Supplemental Tables S1–S3). Using the five general classes of land use and the 13 specific variables, we used a total of 17 spatial predictor variables for analyses (Table 1). We quantified percent cover of each predictor variable in each buffer zone. To estimate percentage of

Table 2 Sample size and information

Sample type	Sample information	Total number
All	Total number of blood and liver samples	378 (individuals, $N = 304$)
	Paired blood and liver information	64 (Simultaneous collection postmortem, $N = 20$; blood collected at captures and liver collected postmortem, $N = 44$)
Blood	Total number	206 (individuals, $N = 195$; recaptures, $N = 11$)
	Type of blood collection event	Live captures, $N = 186$; postmortem, $N = 20$
	Total collected in SMMNRA	189 (LAC, $N = 88$; VC, $N = 101$)
	Total collected in OCSA	16
	Total collected outside of SMMNRA and OCSA	1 (SDC)
Liver[a]	Liver samples	172[b] (Independent samples used in analyses, $N = 169$)
	Total collected in SMMNRA	105 (LAC, $N = 39$; VC, $N = 56$, NA = 10)
	Total collected in OCSA	56 (OC, $N = 52$; RC, $N = 1$; SDC, $N = 3$)
	Total collected outside of SMMNRA and OCSA	11 (SBC and SDC: Rehab centers, $N = 9$; Reported dead, $N = 2$)
Spatial data	Available buffer zone data	Blood, $N = 196$; liver, $N = 121$
Mortalities[b]	Known mortality sources	172 (Mange, $N = 70$; Mange status unknown, $N = 16$; HBC, $N = 67$; Other, $N = 16$; NA = 17; Fetal, $N = 2$; Neonate, $N = 1$)
Mange	Number of cases during each season	Dry season, $N = 43$; Wet season, $N = 26$

SMMNRA Santa Monica Mountains National Recreation Area, *LAC* Los Angeles County, *VC* Ventura County, *OCSA* Orange County Study Area, *OC* Orange County, *SDC* San Diego County, *RC* Riverside County, *SBC* Santa Barbara County

[a] Twenty-three percent of these samples were also used in the Riley et al. (2007) study

[b] Anticoagulant data from three individuals were not used in analyses (fetuses, $N = 2$; neonate, $N = 1$)

each land use type within study areas, we created a single minimum convex polygon surrounding all buffer zones for each study area, and then calculated the percentage of each of the 17 land use variables within each study area's polygon (Table 1).

Data analysis

Descriptive statistics are presented as the mean, standard deviation, median, and range because all data were not normally distributed. Anticoagulant prevalence and 95 % confidence intervals for males, females, adults, and juveniles for wet (November 1–April 30) and dry (May 1–October 31) seasons were calculated separately for blood and liver samples. For prevalence calculations based on blood of recaptured animals, only the data from the most recent capture event was used. For spatial analyses using buffer zone data, we used only recaptures and post-mortem sampling that occurred a minimum of 4 months apart because ARs in blood are expected to decay by this time from an initial exposure (Eason et al. 2002; Erickson and Urban 2004; Vandenbroucke et al. 2008). Consequently, these successive samples of individuals are effectively independent measures of an exposure event, avoiding inflated values caused by multiple recaptures. For a subset of animals ($N = 64$), we had both liver and blood results (Table 2). For this group, we combined the AR residue data for both tissue types to calculate the anticoagulant exposure overall, and 95 % confidence intervals as well as range, mean and median number of compounds detected per individual.

We used 11 different measures of AR exposure for liver samples, and one measure for blood samples (Supplemental Table S4). For liver samples, we evaluated total exposure as presence or absence of any compound as well as individual exposure to each of the four most commonly observed individual compounds (brodifacoum, bromadiolone, diphacinone, and difethialone). We also measured the amount of AR exposure as the total residue concentration in parts per million (ppm) of all compounds detected ("total residues"), as well as separately for each of the four most commonly detected individual compounds. Finally, we used the total number of compounds detected (0–7). Using blood results, we evaluated total exposure only because the majority of detections for ARs in blood were diphacinone, and the total concentration of ARs was quantifiable for less than 10 % of samples tested (24 % of positive samples).

We evaluated risk factors for AR exposure using three types of generalized linear models (GLM). For presence/absence, we used a logistic regression to evaluate risk factors for total exposure measured using blood and liver,

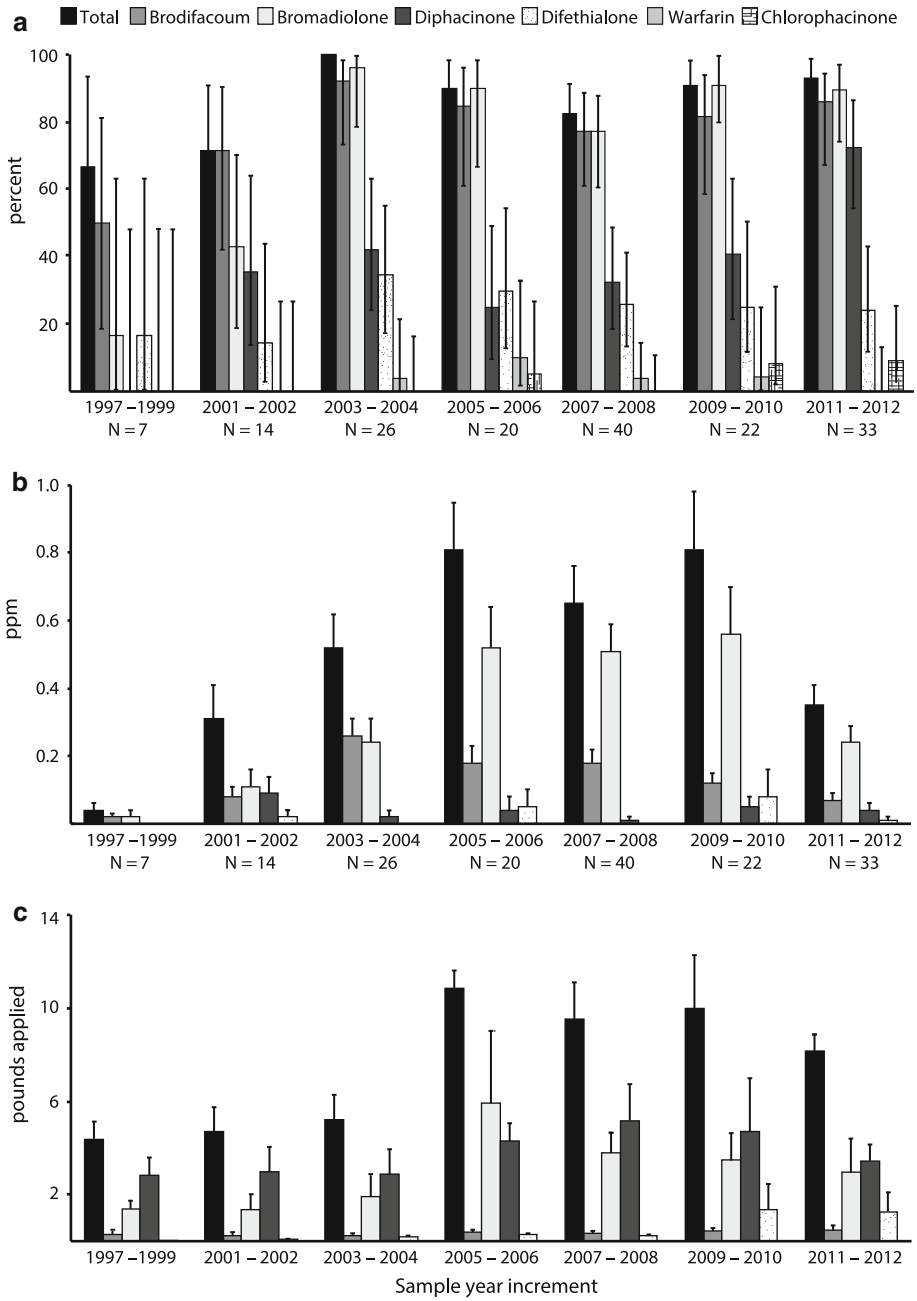

Fig. 2 AR data across 2–3 year time increments. **a** Exposure prevalence overall and by compound per 2–3 year increment. *Error bars* represent 95 % confidence intervals. **b** Concentrations detected per 2–3 year increments. *Error bars* represent standard errors. Warfarin, chlorophacinone, and coumachlor were rarely detected, and if so, were detected at above LOD levels (with the exception of chlorophacinone from 2006 to 2006 when 0.03 ppm was detected). Although lower concentrations of compounds were detected in 2011–2012, the difference, in comparisons with sample years from 2003 to 2010 was not significant. **c** Reported pounds of each compound applied per year increment in the three primary study area counties. *Error bars* represent standard errors. Los Angeles and Ventura Counties are represented across all years and Orange County data was included from 2006 to 2010

and separately, for exposure to brodifacoum, bromadiolone, diphacinone, and difethialone based on liver samples. We used a log-linear GLM to evaluate risk factors for the amount of exposure, both overall using total residues, and using residue concentrations for each of the four most commonly detected compounds in liver tissue. Two of 169 individuals from OCSA had outlier residue concentrations of greater than two standard deviations above the mean, one for difethialone and the other individual for bromadiolone. These individuals were excluded from concentration analyses for these specific compounds and for total residue analyses because preliminary analyses indicated that they dominated model results. We used a Poisson regression to evaluate risk factors for exposure to multiple compounds (0–7) for liver exposure data.

For each model type, we first performed univariate analyses to identify potential predictors, or risk factors, of exposure (Supplemental Table S4). We tested land use categories within each individual buffer zone, study area (SMMNRA, OCSA), sex (male, female), age class (adults $\geq$2; juveniles $<$2 years), age (in years), and season (wet, dry). For each age dataset, we performed separate analyses to avoid potential confounding effects. To evaluate the change in detection rates over time, animals were grouped

into 2–3 year increments depending on the number of animals sampled yearly such that in all time increments, $N \geq 7$ ($N = 23$; Fig. 2). Only four liver samples were collected during 1997–1999, so this time increment was excluded from temporal analyses.

Next, we performed multivariate GLMs to examine the influence of particular predictor variables on AR exposure while controlling for all other significant variables. Variables in the multivariate GLMs were selected by backward stepwise selection using Akaike's Information Criterion (AIC) for model selection. We report the strongest models with ΔAIC values ≤2 (Burnham and Anderson 2002). We report β, the standard error of β, and 95 % confidence intervals for β. A positive β indicates a positive association between the predictor and the exposure outcome, while a negative β indicates a negative association.

We also used logistic regression to examine anticoagulant exposure measures as predictors for notoedric mange. Our predictor variables for these analyses included the 11 anticoagulant exposure measures and the 17 land use predictors. Analyses were performed as above with univariate models followed by multivariate analyses. We also examined the association between notoedric mange and anticoagulant exposure using Fisher's exact tests to evaluate the number of compounds detected (≥2, ≥3, and ≥4) and the threshold value of total residues ≥0.05 ppm suggested by Riley et al. (2007). To further examine the potential relationship between mange and different levels of AR residues, we plotted the proportion of animals exposed to a range of anticoagulant residue concentrations, for animals with and without mange (Fig. 3). For animals with mange, we observed an increase in the proportion exposed to a residue range of 0.25–0.49 ppm. Consequently, we also used a Fisher's exact test to evaluate the association between mange and total residues ≥0.25 ppm. Next, we used a Kolmogorov–Smirnov test to evaluate the difference in the distribution of residue concentrations in bobcats that died with mange compared with those that died without mange. Finally, we used a Wilcoxon-rank sum test to evaluate the difference in median residue concentrations between the two groups.

Because commonly used methods of correction for multiple tests have been described as overly conservative with a higher probability of generating Type II errors in comparison with Type I errors (Moran 2003), we did not correct for multiple tests. Thus, all statistical tests were considered significant when $\alpha \leq 0.05$, but some of these may represent false positives. All statistical analyses were performed in the program R (R Development Core Team 2011).

When data were unavailable for sex (liver, $N = 18$; blood, $N = 2$), age class (liver, $N = 25$; blood, $N = 3$), year sampled (liver, $N = 7$), season sampled (liver, $N = 7$), or mange status ($N = 13$), AR results for those individuals were excluded from prevalence estimates and statistical analyses requiring these data. We also excluded exposure results from statistical analyses for livers from two fetuses (one from each study area), and a liver from a 1 day-old kitten because their exposure was likely not independent from that of their mother.

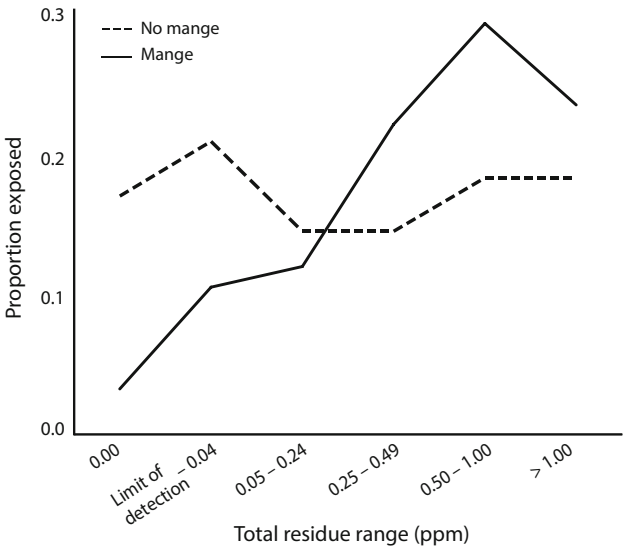

Fig. 3 The proportion of bobcats that died with and without severe mange when exposed to a range of total anticoagulant residues (ppm). The proportion of mange cases, compared with bobcats without mange, increases in the range of 0.25–0.49 ppm, and thus we investigated the relationship between mange and total residues ≥0.25 ppm. The limits of detection vary by compound. For brodifacoum and bromadiolone, the detection limits were 0.05 ppm, whereas the detection limits of chlorophacinone, diphacinone, and difethialone are 0.25 ppm

Results

Prevalence of exposure

Eighty-eight percent of liver samples had 1–5 AR compounds (Table 3; mean = 2.32, median = 2.00). The range of total residues detected in liver was 0.00–5.81 ppm (mean = 0.59, SD = 0.80, median = 0.40). The compounds most frequently detected were second-generation bromadiolone, brodifacoum, and difethialone, and first-generation diphacinone. Mean values for the four most commonly detected compounds were: brodifacoum, 0.14 ppm (SD = 0.20); bromadiolone, 0.38 ppm (SD = 0.55); difethialone, 0.04 ppm (SD = 0.31); diphacinone, 0.03 ppm (SD = 0.12). Brodifacoum and bromadiolone were the two most frequently detected ARs in liver samples (Fig. 4) and were detected approximately twice as frequently as difethialone or diphacinone. Warfarin and

Table 3 Proportion (Prop.) and 95 % confidence intervals of anticoagulant exposure across the study areas

Variable	Group	Liver			Blood		
		N	Prop.	95 % CI	*N*	Prop.	95 % CI
	All	169	0.88	0.82–0.92	195	0.39	0.32–0.46
Sex	Female	77	0.88	0.78–0.94	86	0.38	0.28–0.50
	Male	74	0.89	0.79–0.95	107	0.40	0.31–0.50
Age class	Adult	107	0.91	0.83–0.95	127	0.40	0.32–0.49
	Juvenile	37	0.86	0.70–0.95	65	0.37	0.26–0.50
Season	Wet	96	0.90	0.81–0.95	139	0.32	0.25–0.41
	Dry	66	0.89	0.81–0.94	56	0.55	0.42–0.68

Prevalence is partitioned by sample type, sex, age class, and season. When information on sex, age class, or season collected was not available, those data were not included in the proportion estimates, and so data partitioned by sex, age class, and season may not sum to the total number of blood or liver samples

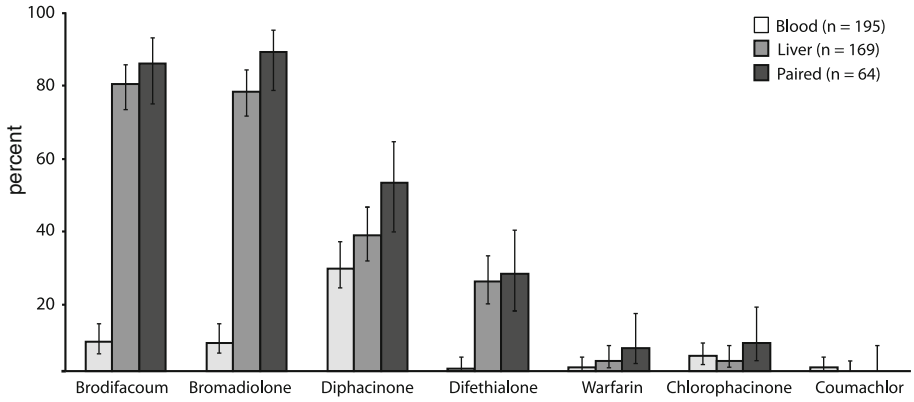

Fig. 4 Detection prevalence of each anticoagulant compound in the liver, blood, and for a subset of individuals, paired blood and liver tissue results are provided

chlorophacinone were rarely detected and coumachlor was not detected in liver samples. Seventy-seven percent of all bobcats and 87 % of those exposed showed the presence of ≥2 compounds in the liver.

In contrast, 39 % of blood samples tested positive for ARs (Table 3), most frequently to one compound (76 % of positives), but ranging from 0 to 4 compounds (mean = 0.53 compounds, median = 0.00). The total residues detected in blood ranged from 0 to 0.16 ppm (mean = 0.002, SD = 0.01, median = 0.00). Diphacinone, the most commonly detected compound in blood, was detected more than three times as frequently as brodifacoum or bromadiolone (Fig. 4). For animals with both blood and liver samples ($N = 64$), 92 % were exposed, most frequently to three or more compounds (median = 3.00, mean = 2.61, range 1–5).

Percent exposure was similar across sexes and age classes using liver or blood samples (Table 3). Sixty-six individuals were aged by cementum annuli (age range: 0–12 years). Fourteen individuals had age class data estimated during capture, and cementum annuli data collected postmortem. We used these paired data to test the accuracy of our age class estimations during captures and found we assigned correct age classes to 12 of 14 individuals. We did not detect a significant association between age and AR exposure measures.

Exposure did not vary by season when tested using liver samples (Table 3). In contrast, based on blood results, animals were significantly more likely to be exposed during the dry season [Odds ratio (OR) = 2.58] compared with the wet season (Tables 3, 4). Overall we detected 72 % more exposure in blood during the dry season than during the wet season with 32 % exposure detected during the wet season, and 55 % exposure detected during the dry season.

We examined exposure prevalence over time in liver samples and found exposure to exceed 67 % for all years, indicating high exposure prevalence throughout the study (Fig. 2a). Exposure rates varied for each of six compounds across sampling increments (Fig. 2a). Overall exposure was highest during 2003–2004 and 2011–2012. There was significantly less exposure overall and to bromadiolone in 2001–2002 compared with other years (Table 5; Fig. 2a). Diphacinone exposure was significantly greater in 2003–2004 and 2011–2012 compared with other time increments (Table 5; Fig. 2a). However, both total and

Table 4 Results of Fisher's exact tests for parameters that were significant during univariate GLM analyses

Sample type	Parameter	Comparison	Odds ratio	95 % confidence interval	*P*
Liver	Total residues ≥0.05 ppm	Severe mange versus no mange	4.00	1.67–10.48	<0.001
	Total residues ≥0.25 ppm	Severe mange versus no mange	3.16	1.51–6.84	<0.001
	Exposure to ≥2 AR compounds	Severe mange versus no mange	7.27	2.55–25.70	<0.001
	Exposure to ≥3 AR compounds	Severe mange versus no mange	2.11	1.06–4.23	0.023
	Exposure to ≥4 AR compounds	Severe mange versus no mange	3.98	1.54–11.26	0.002
Blood	Exposure detected	Dry season versus wet season	2.58	1.31–5.14	0.004
	Exposure detected	Capture event versus mortality	5.55	1.80–20.49	0.001
	Exposure detected	Capture event versus vehicle mortality	∞	1.00–∞	0.006

Table 5 Significant predictors of presence or absence of exposure in blood and liver

Outcome	Predictors of exposure		β	β SE	β 95 % CI	*P*
Total exposure (blood)	Dry season		0.95	0.32	0.31–1.56	0.003
	Crops, pastures, orchards and vineyards		4.85	2.08	0.98–9.21	0.015
	Horse ranches		88.75	36.10	21.90–166.11	0.011
	Other agriculture		15.46	7.29	1.63–30.67	0.029
	Water transfer and storage facilities		93.63	36.16	29.58–174.10	0.006
	Golf courses		15.69	7.75	0.50–30.88	0.043
	Multifamily high-density residential		9.47	3.56	2.49–16.44	0.008
	Single-family high-density residential		1.87	0.88	0.14–3.60	0.035
	Total residential		4.36	1.80	1.01–8.02	0.016
	Total commercial/industrial		4.42	1.84	0.81–8.02	0.016
	Total altered open		17.17	6.63	2.43–49.17	0.010
	Total residential		2.61	0.82	1.01–4.20	0.001
	Natural		−3.41	0.68	−4.74 to −2.09	<0.001
Total exposure (liver)	Single-family high-density residential		7.58	3.45	0.81–14.34	0.028
	Total residential		6.05	2.29	1.56–10.53	0.008
	Year (2011–2012 reference)	2001–2002	−2.72	1.18	−5.03 to 5.51	0.021
Brodifacoum exposure	Crops, pastures, orchards and vineyards		−5.62	2.67	−10.87 to −0.38	0.036
	Single-family high-density residential		6.19	2.36	1.56–10.82	0.009
	Total residential		6.68	1.90	2.95–10.41	<0.001
Bromadiolone exposure	Year (2011–2012 reference)	2001–2002	−1.54	0.67	−2.91 to −0.17	0.022
Diphacinone exposure	Single-family high-density residential		2.31	1.12	0.11–4.51	0.039
	Total residential		2.07	0.99	0.14–4.01	0.035
	Year (2011–2012 reference)	2001–2002	−1.46	0.70	−2.83 to −0.09	0.036
		2005–2006	−1.67	0.62	−2.98 to −0.52	0.005
		2007–2008	−1.30	0.48	−2.34 to −0.42	0.005
		2009–2010	−0.94	0.56	−2.26 to −0.01	0.048

Only results from statistically significant univariate analyses are shown

bromadiolone residue concentrations detected were greatest between 2005 and 2010, although the variation in residue concentrations across time was not significant (Fig. 2b). These years included samples from OCSA, where significantly greater bromadiolone residues were detected (Table 6; Fig. 5). Although the residue concentrations we detected in 2011–2012 were lower for all compounds the differences in overall exposure and residue concentrations were not significant. The apparent decrease in residue concentrations is the result of having OCSA samples, where bromadiolone residues were significantly higher for the years 2006–2010 (see below and Supplemental Fig. S1b). Further, the decrease in total and bromadiolone residues mirrors the County reports we compiled of the amount of rodenticide (in pounds) applied (Fig. 2c, Supplemental Fig. S1c). In blood samples, we did

Table 6 Significant predictors of AR residue concentrations, total compounds detected, notoedric mange, and exposure detected in blood at the time of capture versus mortality

Outcome	Predictor variables		β	β SE	β 95 % CI	*P*
Total concentration	Golf courses		5.88	1.01	3.90–7.85	<0.001
	Single-family high-density residential		1.24	0.46	0.34–2.13	0.008
	Total altered open		5.66	0.98	3.74–7.58	<0.001
	Total residential		1.31	0.44	0.44–2.17	0.004
	Natural		−1.20	0.35	−1.88 to −0.52	0.001
	Study area: OCSA		0.74	0.17	0.41–1.08	<0.001
Brodifacoum concentration	Office/retail		5.13	1.17	2.84–7.42	<0.001
	Golf courses		4.16	1.45	1.30–7.20	0.006
	Single-family high-density residential		1.31	0.54	0.25–2.37	0.017
	Total altered open		4.28	1.42	1.49–7.07	0.003
	Total residential		1.31	0.53	0.28–2.34	0.014
	Natural		−0.93	0.42	−1.75 to −0.11	0.029
	Study area: OCSA		0.58	0.22	0.11–0.96	0.014
Bromadiolone concentration	Mixed commercial/industrial		5.10	1.29	2.57–7.63	<0.001
	Golf courses		7.45	0.95	5.59–9.30	<0.001
	Multifamily high-density residential		1.58	0.76	0.09–3.08	0.040
	Single-family high-density residential		1.38	0.52	0.36–2.39	0.009
	Total commercial/industrial		1.43	0.57	0.31–2.55	0.014
	Total altered open		7.16	0.92	5.36–8.96	<0.001
	Total residential		1.38	0.51	0.38–2.39	0.008
	Natural		−1.45	0.40	−2.24 to −0.67	<0.001
	Study area: OCSA		1.03	0.21	0.61–1.45	<0.001
Diphacinone concentration	Mixed commercial/industrial		8.90	3.20	2.62–15.17	0.006
Total compounds	Single-family high-density residential		0.80	0.32	0.16–1.43	0.014
	Total residential		0.92	0.29	0.35–1.49	0.002
	Natural		−0.47	0.22	−0.90 to −0.03	0.036
	Year (2011–2012 reference)	2001–2002	−0.57	0.24	−1.04 to −0.10	0.018
Mange	Exposure		1.90	0.78	0.37–3.43	0.015
	Brodifacoum exposure		1.74	0.52	0.71–2.76	0.001
	Brodifacoum concentration		1.84	0.89	0.08–3.59	0.040
	Difethialone exposure		1.16	0.39	0.39–1.92	0.003
	Total compounds		0.56	0.15	0.26–0.85	<0.001
	Total residential		2.38	1.01	0.39–4.37	0.019
Mortality	Exposure (blood)		1.72	0.54	0.67–2.78	0.001

Only results from statistically significant univariate analyses are shown

not detect a trend of exposure prevalence across sampling years.

Two fetal bobcats were exposed to anticoagulant compounds. One animal was exposed to two compounds (brodifacoum and diphacinone) and the other was exposed to five compounds (brodifacoum, bromadiolone, diphacinone, difethialone, and chlorophacinone). For both fetuses, all compounds detected were above LOD but not quantifiable. The mother of the fetus with five compounds was also tested for exposure and had quantifiable levels of brodifacoum (0.32 ppm), bromadiolone (0.58 ppm) and was positive for difethialone, diphacinone, and chlorophacinone.

Spatial correlates of exposure

Exposure prevalence measured using liver tissue did not significantly differ between SMMNRA (89, 95 % CI 81–94; $N = 104$) and OCSA (84, 95 % CI 71–92; $N = 55$) (Fig. 5). The mean total residues were significantly greater in OSCA (Fig. 5; Table 6) even with two outliers removed (OCSA, 0.84 ppm; SMMNRA, 0.40 ppm). Brodifacoum and bromadiolone were each detected at significantly greater concentrations in liver tissue collected in OCSA (0.21 and 0.63 ppm) compared with SMMNRA (0.12 and 0.22 ppm) (Table 6).

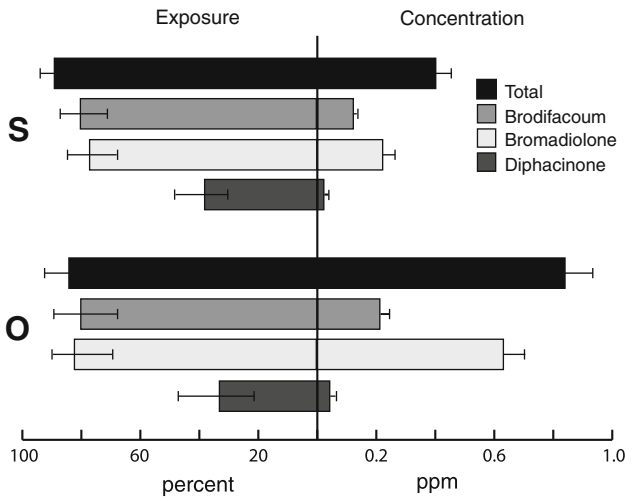

Fig. 5 *Left* Percent total exposure and exposure to individual compounds in SMMNRA (S) and OCSA (O). Bars represent 95 % confidence intervals. *Right* Total residue concentration and residue concentrations for each compound. *Bars* represent standard errors

Landscape variables were important predictors of exposure in both blood and liver samples (Tables 5, 6). Percent natural area in each individual buffer zone was negatively associated with multiple measures of exposure in blood and liver (Table 5). Golf courses and total altered open area were positively associated with exposure in blood (Table 5) and with total residues and the concentrations of bromadiolone and brodifacoum in liver (Table 6).

In terms of the non-residential urban and agricultural areas, all three more specific agricultural categories (Table 1, Supplementary Table S2) and total agricultural area were positively associated with exposure in blood (Table 5). However, brodifacoum exposure was negatively associated with the category comprised of open, active agriculture (crops, pastures, orchards, and vineyards; Table 5), and given that second-generation ARs are restricted for use indoors and within 100 m from human structures, this negative association is not surprising. Commercial and industrial areas were positively associated with bromadiolone and diphacinone concentrations in liver samples (Table 6). Water storage and transfer facilities and total commercial and industrial areas were positively associated with exposure in blood samples (Table 5). Office and retail area was positively associated with brodifacoum concentration in liver samples (Table 6).

Residential areas, by far the most common type of development in these study areas (22 %, SMMNRA and 23 %, OSCA), were frequently positively associated with AR values observed in both blood and liver samples (Tables 5, 6). In particular, single-family high-density residential area was among the most frequent land use type to have positive associations with anticoagulant exposure, and was significant for 8 of 11 anticoagulant exposure models tested (Tables 5, 6). In terms of broader measures, single-family high-density residential area was positively associated with overall exposure in blood and liver and the total number of compounds and total residues in liver samples. For specific compounds, single-family high-density residential was also positively associated with brodifacoum and diphacinone exposure and brodifacoum and bromadiolone concentrations in liver. Total residential area was also frequently important in univariate models. In terms of exposure, total residential area was associated with exposure in blood and liver and exposure to brodifacoum and diphacinone in liver. Total number of compounds, total residues, and liver concentrations of brodifacoum and bromadiolone were also positively associated with total residential area.

Multivariate models were significant for five measures of ARs in bobcats (Table 7). In terms of exposure, year and total residential area were important for diphacinone, and for total exposure, percent natural area (the reciprocal of percent development) was significant, along with season. For the amount of ARs detected in liver tissue, the best-fit model included golf courses, single-family high-density residential, and OCSA as the most important risk factors. For brodifacoum concentration detected in liver tissue, office and retail, single-family high-density residential, and total altered open space were the three most important predictors of residue load. Finally, mixed commercial and industrial, golf courses, single-family high-density residential, and OCSA were the most important predictors of total bromadiolone concentration in liver tissue.

Anticoagulants and notoedric mange

The median total residues for bobcats with mange was 0.52 ppm (mean = 0.65, SE = 0.06), while for bobcats that died without mange, the median total residues was 0.24 ppm (mean = 0.53, SE = 0.09), a significant difference ($W = 2141.00$, $P = 0.005$). The distribution of residue concentrations within the two groups also differed significantly ($D = 0.28$, $P = 0.004$). The median number of compounds observed was 3 (mean = 3.00) in bobcats with mange and 2 (mean = 2.00) for bobcats without mange. Sixty-four percent of bobcats without mange tested positive for ≥2 compounds, while 93 % of bobcats with mange tested positive for ≥2 compounds.

Severe mange was positively associated with anticoagulant exposure, brodifacoum exposure, difethialone exposure, brodifacoum concentration, and the total number of compounds detected. In terms of land use, mange was positively associated with total residential area, but this was the only significant land use predictor (Table 6). The mean total residential area in mange bobcat buffer zones was 32.2 % (SD = 18.61, median = 29.39) compared with a mean of 23.3 % for bobcats without mange

Table 7 Results of the best-supported statistically significant multivariate model analyses for anticoagulant exposure and mange

Outcome	Best-supported model	Predictor variables	β	β SE	β 95 % CI	*P*
Total exposure (blood)	Season + natural	Dry season	0.71	0.35	0.02–1.40	0.043
		Natural	−3.29	0.68	−4.62 to −1.95	<0.001
Diphacinone exposure	Total residential + year	Total residential	2.57	1.12	0.37–4.77	0.022
		2001–2002	−1.62	0.82	−3.23 to −0.02	0.048
		2003–2004	−1.42	0.69	−2.77 to −0.62	0.040
		2005–2006	−2.11	0.80	−3.68 to −0.55	0.008
		2007–2008	−1.78	0.65	−3.06 to −0.50	0.006
		2009–2010	−1.43	0.72	−2.84 to −0.01	0.048
Total concentration	Golf courses + single-family high-density residential + study area	Golf courses	3.91	1.06	1.84–5.98	<0.001
		Single-family high-density residential	0.99	0.43	0.15–1.82	0.022
		OCSA	0.69	0.20	0.30–1.07	0.001
Brodifacoum concentration	Office/retail + single-family high-density residential + total altered open	Office/retail	4.49	1.09	2.34–6.63	<0.001
		Single-family high-density residential	1.22	0.55	0.15–2.29	0.027
		Total altered open	3.88	1.55	0.85–6.91	0.013
Bromadiolone concentration	Mixed commercial/industrial + golf courses + single-family high-density residential + study area	Mixed commercial/industrial	3.48	0.99	1.55–5.42	0.001
		Golf courses	5.69	0.93	3.87–7.51	<0.001
		Single-family high-density residential	1.24	0.42	0.42–2.06	0.004
		OCSA	0.90	0.25	0.42–1.38	<0.001
Mange	Difethialone exposure + brodifacoum exposure	Brodifacoum exposure	1.54	0.53	0.49–2.58	0.004
		Difethialone exposure	0.93	0.40	0.14–1.72	0.021

(SD = 19.60, median = 19.05). In the multivariate model, after controlling for multiple AR parameters and land use, brodifacoum and difethialone exposure remained significant predictors of severe mange while land use was not (Table 6). We found a strongly significant association between mange and total residues ≥0.05 ppm and total residues ≥0.25 ppm (Fig. 3; Table 4). Bobcats that were exposed to ≥0.05 ppm were 4.0 times (95 % CI 1.67–10.48) more likely to die with severe notoedric mange than without, while those exposed to ≥0.25 ppm were 3.2 times (95 % CI 1.51–6.84) more likely to die with severe mange. Additionally, we observed a strong association between exposure to ≥2 compounds and severe mange (Table 4). Specifically, bobcats were 7.3 times (95 % CI 2.55–25.70) more likely to die with severe mange than without if they were exposed to 2 or more AR compounds. There were also significant associations between mange and exposure to ≥3 and ≥4 compounds (Table 4).

Anticoagulants and mortality

Anticoagulant exposure detected in blood was significantly more frequent in samples collected postmortem compared with samples collected antemortem (Tables 4, 6). In 75 % of blood samples collected postmortem ($N = 20$), we detected at least one AR compound. When blood samples collected at the time of mortality were excluded from blood AR prevalence estimates, we detected a 34 % exposure prevalence in blood samples collected at the time of animal capture ($N = 175$) compared with 39 % overall ($N = 195$). For blood samples collected at the time of mortality, ARs were detected in 77 % of bobcats that died of notoedric mange ($N = 13$), 100 % of bobcats that died of vehicle collision ($N = 5$), and one bobcat that died of starvation after a wildfire. Three bobcats that died of mange, one from a control action, and another that died of unknown cause did not have detectable ARs in their blood.

Discussion

We documented widespread exposure of bobcats to first- and second-generation ARs in two southern California areas. Bobcats are obligate carnivores that consume a wide range of small mammals (Anderson and Lovallo 2003) including mice, rats, and gophers (Fedriani et al. 2000;

Riley et al. 2010) that are frequent targets of pest control activities within SMMNRA (Morzillo and Mertig 2011a, b; Morzillo and Schwartz 2011; Bartos et al. 2012) and elsewhere (Morzillo and Mertig 2011b). Given that bobcats are obligate carnivores, it is very unlikely that they consume rodent baits directly. Thus, bobcat exposure to ARs is predominantly, if not entirely, secondary through prey consumption. Exposure rates and compounds detected varied considerably by sample type, but in individuals having blood and liver data (and therefore most comprehensively sampled), we detected an AR exposure rate of 92 % across the study areas, with animals most frequently exposed to three or more compounds. These findings are among the highest reported prevalence rates for AR exposure in a nontarget predatory species (e.g. Shore 2003; Fournier-Chambrillon et al. 2004; Riley et al. 2007; Walker et al. 2008; Gehrt and Riley 2010; Elmeros et al. 2011; Gabriel et al. 2012; Sánchez-Barbudo et al. 2012). Additionally, the combined liver and blood results indicate that exposure prevalence and exposure to certain compounds, specifically diphacinone, may be underestimated with liver samples alone (Fig. 4). We detected exposure to multiple AR compounds in two fetal bobcats, the first such cases, to our knowledge, reported for any wildlife species in a natural population. These data, including individuals caught multiple times more than 4 months apart, indicate multiple exposure events and suggest the potential for chronic exposure to ARs that can begin during prenatal development.

There are no toxicokinetic studies (the movement of toxic substances within the body) of ARs in wildlife, however, hepatic half-lives for ARs are reported across multiple species to be longer than plasma half-lives, particularly for second-generation ARs (Kamil 1987; Robben et al. 1998; Petterino and Paolo 2001; Vandenbroucke et al. 2008). The toxicokinetics of secondary AR exposure is more complex because the movement of the residues in both the primary and secondary consumer must be considered (Erickson and Urban 2004). Thus, we are limited in our ability to interpret bobcat AR exposure results with respect to dose and time since exposure using either blood or liver sample data. However, because we most frequently detect diphacinone in blood despite its shorter plasma half-life than second-generation ARs (Erickson and Urban 2004), diphacinone may be the compound that bobcats encounter most frequently in SMMNRA.

Risk factors for exposure

Exposure detected using liver tissue was high throughout the course of the 16-year study, ranging from 67 to 100 % for each 2- to 3-year time period, indicating high prevalence AR exposure in bobcats since at least 1997. Our samples indicated an increase in overall exposure both in prevalence and residue concentrations since 2002. We detected significant increases in total AR exposure, bromadiolone exposure, and total number of detected compounds. With the exception of diphacinone, overall exposure prevalence and exposure to individual compounds appears to have been relatively constant from 2003 to 2012. Total residues and bromadiolone residues were highest from 2005 to 2010, the time increments for which OCSA samples were available, which appears to reflect the degree of bromadiolone use in Orange County. Diphacinone exposure also increased in frequency from 1997 to 2012, reaching a high in 2011–2012. Despite this increase, the quantity applied in each county as reported to Department of Pesticide Regulation does not appear to have significantly changed over the course of the study (Fig. 2c, Supplemental Fig. S1c). Thus, increased diphacinone exposure may be the result of increased use of the compound in residential areas by homeowners and pest control companies that are not required to report amounts of ARs applied annually. In fact, single-family high-density and total residential area were important predictors of diphacinone exposure. Diphacinone is a first-generation compound and is considered to pose less risk to nontarget wildlife than the more toxic second-generation ARs (Erickson and Urban 2004), although first-generation ARs still pose a risk for toxic effects to wildlife, and secondary exposure can be a direct source of mortality for some species (Littrell 1988; Stone et al. 1999; Riley et al. 2003). Further, the degree to which there are additive or interactive effects between diphacinone and second generation ARs is unknown.

As measured in blood, we detected more than twice as much AR exposure during the dry season compared with the wet season. In southern California, the dry season coincides with peak rodent activity (Meserve 1976), and residents in the region are known to use ARs to target rat, mice, squirrel, and gopher populations (Morzillo and Schwartz 2011; Bartos et al. 2012). Although we detected no seasonal differences in exposure in liver samples, the long hepatic half-lives of second-generation ARs likely obscured our ability to detect seasonal differences. Additionally, because second-generation ARs may persist in small mammal species from 90 to 135 days after removal of poison baits, poisoned small mammals may remain a continuing source of exposure for predatory species long after the end of poisoning programs (Murphy et al. 1998; Sage et al. 2008).

Because an accumulated risk of exposure may occur with bobcat age, and female bobcats have smaller home ranges and are less likely to use urban areas compared with males (Riley et al. 2003, 2010), we expected to detect demographic differences in AR exposure prevalence and residue concentrations. However, neither age nor sex significantly influenced exposure in our study areas. Within

our study areas, the high prevalence of exposure may have diminished our ability to detect demographic differences. Further, the movement patterns and relatively high mobility of some rodent species may lead to AR exposure in even those individuals that avoid the use of urban areas (Riley et al. 2010). For example, wood mice (*Apodemus sylvaticus*) and house mice (*Mus domesticus*) were found exposed to multiple AR compounds in Northern Ireland even though they were sampled in agricultural areas where ARs were not in use (Tosh et al. 2012). Thus, movement of poisoned prey between areas may occur where AR control efforts differ (Tosh et al. 2012). The risk of secondary AR exposure in predatory species, therefore, may not be limited to areas where ARs are in use. As a result, even individuals that use urban areas less, such as female bobcats and not yet dispersed young animals, may still be at high risk of AR exposure.

Spatial predictors of exposure

The association between AR exposure and specific land use types likely reflects the degree of AR use in those areas. Previous studies have found an association between anthropogenic development and AR exposure in nontarget wildlife. For example, 95 % ($N = 74$) of wildlife carcasses sampled across California from 1994 to 1999 with exposure to ARs were reported to have been collected in areas with significant urban development (Hosea 2000). However, there was no specific information about the type and intensity of urban development where individuals were sampled. Other previous studies in these areas found a positive association between total AR concentrations and the percent of bobcat (Riley et al. 2007) and mountain lion (Beier et al. 2010) radio-telemetry locations in areas affected by anthropogenic development, including areas classified as altered open and areas of more intense urban development (e.g. composite residential, commercial, and industrial areas).

Single-family high-density residential (5–10 housing units/ha) and golf courses were among the most frequent risk factors for various measures of AR exposure, despite comprising a relatively small percentage of the study areas (15.9 and 1.4 %), suggesting their importance as a risk factor for AR exposure and toxicant loads. In a recent study in two southern California areas (SMMNRA, Bakersfield), residents in single-family high-density structures were the most likely to use ARs to control pest populations compared with those in multifamily or single-family low-density structures (Morzillo and Schwartz 2011). Residential AR use was highest in areas in close proximity to open areas, whether natural or altered open, compared with residential areas farther away from open spaces. Golf courses and other altered open spaces in the study areas are typically surrounded by, or very near to, single-family housing units. Of 21 golf courses in our study areas, 19 are bordered on at least 1 side by single-family high-density residential areas. Because residential AR use may be elevated in areas with altered open space in close proximity (Morzillo and Schwartz 2011), the association between AR exposure and altered open areas may also be the result of increased AR use in the single-family residential areas adjacent to golf courses. In OSCA, where bobcats had greater brodifacoum and bromadiolone residue loads, the mean percent of golf courses in bobcat buffer zones was nearly five times greater than in SMMNRA (0.6 vs. 2.7 %), potentially contributing to increased residue loads in OCSA. Although the residential and altered open types of urban development comprise a relatively small proportion (<25 %) of the study areas, Morzillo and Schwartz (2011) suggested a small degree of AR use in residential areas can lead to increased exposure risk for wildlife. Both bobcats (Riley et al. 2010) and coyotes (Gehrt and Riley 2010) have been observed to routinely utilize residential and altered open areas such as golf courses, increasing their probability of exposure to ARs if the compounds are regularly used there or nearby.

Although percent natural habitat was negatively associated with AR exposure and total residues, four bobcats whose buffer zones comprised 100 % natural habitat were found exposed to ARs. These data indicate that ARs may also affect wildlife living solely within protected park areas. Both of the individuals with bromadiolone residues were radio-collared during ongoing NPS research in SMMNRA, and their documented home ranges did not extend beyond protected park boundaries (Riley et al. NPS unpubl. data). Previous NPS research on coyote utilization of urban areas found that even animals with the lowest urban association died directly from AR toxicosis (Riley et al. 2003). A recent study on fishers (*Martes pennanti*), a remote forest carnivore in protected undeveloped parkland in northern California, found 79 % of fishers exposed to ARs and that four died directly of anticoagulant toxicosis (Gabriel et al. 2012). Gabriel et al. (2012) suggested illegal marijuana cultivation in remote areas could have been the source of ARs. Within SMMNRA, illegal marijuana cultivation also occurs, so this may also contribute to AR exposure for animals that reside entirely in protected park areas.

Consequences of exposure

Although the prevalence of AR exposure was very high at 92 %, AR exposure alone does not appear to be a significant source of direct mortality for bobcats. At present, there are few cases of AR toxicosis in bobcats documented in the literature. None of the bobcats in OCSA died directly

of anticoagulant toxicity, and in a broader study of poisoning cases of wildlife in California, Hosea (2002) observed clinical signs consistent with anticoagulant toxicosis in two bobcats, one of which was an individual from SMMNRA (Riley et al. 2007). In Marin County, a radio-collared bobcat died of anticoagulant toxicity; chlorophacinone was detected in the liver tissue (Riley 1999). AR exposure was suspected to have caused gastrointestinal bleeding in bobcats that died of severe notoedric mange and were exposed to ARs in several counties in California, though other signs of anticoagulant toxicity were absent (Serieys et al. 2013). Domestic cats are reported to be more tolerant of AR exposure than dog or rodents (Petterino and Paolo 2001; Erickson and Urban 2004). Whether this tolerance is similar for wild felids is unknown, but if so, it may account for the few cases of toxicosis detected. However, felid tolerance to low-grade AR exposure may increase their vulnerability to sublethal toxicosis, or affect their ability to respond to external stimuli such as predators and vehicles (see below).

In SMMRNA, secondary anticoagulant rodenticide exposure was associated with a population decline (Riley et al. 2007) and a genetic bottleneck (Serieys et al. 2014) that occurred due to notoedric mange. Mange and vehicle collisions are the primary sources of mortality for bobcats in our two southern California study areas (Riley et al. 2010). Notoedric mange is now documented in eight counties in northern and southern California. Across all of these areas, animals that died of mange were found to be exposed to ARs whenever tests were conducted (Serieys et al. 2013; Clifford, pers.comm.). Interestingly, 65 % of severe bobcat mange cases observed in our study areas during this 16-year period occurred during the dry season, coincident with increased AR exposure detected in blood samples. Sixty-nine of 70 bobcats that died with severe mange (covering >70 % of their body) were exposed to ARs. We detected a strong association between exposure to ≥2 compounds and notoedric mange. Detection of multiple compounds in a single individual suggests multiple exposure events since rodenticide baits sold in California are each formulated with a single compound. Thus, we suggest that a single anticoagulant exposure event itself may not increase bobcat susceptibility to mange, but rather repeated exposure events may be an important predictor of potential sublethal effects such as increased susceptibility to mange.

Severe mange in free-ranging wildlife and domestic animals is often associated with decreased immune competence (Pence and Ueckermann 2002). Humans that are immunocompromised are also more likely to suffer severe, crusted forms of mange due to infestation with a related mite, *Sarcoptes scabiei* (Walton et al. 2004; Roberts et al. 2005). The mode by which anticoagulant rodenticide exposure could compromise bobcat immunity is unknown, although recent studies in humans and laboratory animals have shown therapeutic doses of warfarin to have both immunostimulatory and suppressive effects when administered for ≤30 days (Kurohara et al. 2008; Belij et al. 2012; Popov et al. 2013). Laboratory experiments have shown that interactive effects between sublethal exposure to anticoagulants and other stressors can induce mortality. For laboratory subjects, sublethal anticoagulant doses produced 40–70 % mortality when combined with other stressors, such as frostbite (Jaques 1959). When stressed by shearing and captivity, sheep (*Oves aries*) required lower doses of the first-generation AR pindone to die as a result of anticoagulant toxicosis (Robinson et al. 2005). A potential interaction between the toxic effects of chlorophacinone and a bacterial pathogen, tularemia (*Francisella tularensis)* was described in common voles (*Microtus arvalis*, Vidal et al. 2009). Voles that were infected with *F.tularensis* died at lower doses of chlorophacinone than uninfected voles. Tularemia prevalence was also higher in areas treated with chlorophacinone, and the authors suggested that the AR field treatment may have also facilitated the spread of the disease in the affected vole population.

Sublethal AR exposure may also negatively affect individuals directly. In Denmark, Elemeros et al. (2011) found a negative association between anticoagulant exposure and body condition in weasels (*Mustela nivalis*) and stoats (*Mustela erminea*). A reduced escape response has been observed in rats dosed with ARs (Cox and Smith 1992), and if carnivores secondarily exposed to ARs have a similarly reduced response to threats, they may be more vulnerable to vehicle collisions or predation. Elmeros et al. (2011) found that for both stoats and weasels, those that were sampled after being trapped had significantly lower total AR residue concentrations than those sampled after vehicle collisions and predation events. Although we have a limited sample size ($N = 5$), all animals that died of vehicle collisions for which we collected blood post-mortem had detectable AR residues in their blood (compared with 34 % of captured animals). Thus we speculate that recent AR exposure events may increase bobcat vulnerability to vehicle collision but additional data are needed to test this hypothesis.

Bobcats with severe notoedric mange exhibit altered behavior increasing their susceptibility to other primary sources of mortality. For example, although bobcats are primarily nocturnal, especially in urban populations (Riley et al. 2003), we have observed bobcats with severe mange infestation frequently wandering in urban areas during daylight hours (Riley and Serieys unpubl.data). This shifted activity pattern may increase the risk of being struck by vehicles and vulnerability to other sources of mortality.

Though sample sizes are limited, our findings that AR transfers from mother to offspring suggests consequences

for reproduction in bobcats. Contaminant exposure that interferes with the reproductive success of wildlife populations can lead directly to population declines. We tested two bobcat fetuses, one from each study area and both were exposed to multiple AR compounds with one exposed to five compounds. Reproductive consequences associated with AR exposure in other species have included increased miscarriage, fetal toxicosis, fetal congenital deformities, and decreased sperm counts in humans (Ginsberg and Hirsh 1989), dogs (Munday and Thompson 2003), and sheep (Robinson et al. 2005). In humans, prenatal exposure to first-generation coumarin even at low, therapeutic doses has been associated with central nervous system abnormalities (Ginsberg and Hirsh 1989; Wesseling et al. 2001). Brodifacoum toxicosis was documented in neonatal puppies even though the mother was exposed 4 weeks prior to birth (Munday and Thompson 2003). AR exposure may be an important challenge for population viability in urban areas if chemical contamination causes detrimental effects on reproduction.

Conservation and management implications

Exposure of nontarget wildlife to ARs is increasingly recognized as a widespread conservation issue (Erickson and Urban 2004; US EPA 2008; California Department of Pesticide Regulation 2013) and numerous species have been exposed, sometimes causing direct mortalities (Scheuhammer 1987; Peakall 1992; Eason et al. 2002; Erickson and Urban 2004; Riley et al. 2007; Gabriel et al. 2012). Species that are exposed include federally listed endangered species such as San Joaquin kit foxes (McMillin et al. 2008), bald eagles (*Haliaeetus leucocephalus*, Stone et al. 2003; Salmon 2010), and the Northern spotted owl (*Strix occidentalis caurina*, Erickson and Urban 2004). Indirect mortalities associated with the poisons may also pose an important threat for wildlife populations, particularly those that are re-colonizing parts of their past range. For example, during a recent study of California fishers, which are candidates for protection under the US Endangered Species Act, a lactating female died of anticoagulant toxicosis, which most likely led indirectly to the death of her litter (Gabriel et al. 2012). For threatened populations, exposure to ARs may influence their reproductive success, lead to sublethal and lethal consequences and increase their vulnerability to other sources of mortality.

Although some U.S. States, such as California, are taking steps to increase regulation of the use and the availability of these poisons to consumers, the adequacy of these is unknown. Under current law, second-generation ARs are restricted to indoor use or within 30 m (100 ft) of buildings. In California, the Department of Pesticide Regulation has reduced that distance to a 17 m (50 ft) radius from buildings. However, Tosh et al. (2012) found no relationship between distance from buildings and residue concentrations in two species of mice reflecting the high mobility of the small mammals even after ingestion of ARs. They also detected a contaminated wood mouse (*Apodemus sylvaticus*) 110 m from a building where usage occurred and another 160 m from a building where no usage occurred (Tosh et al. 2012). In residential areas within SMMNRA, residents have reported off-label use of ARs, and use of second-generation ARs up to 100 m from buildings (Bartos et al. 2012). We have observed containers of second-generation ARs in natural areas behind homes at greater than 30 m from a building. Residents who use ARs have also reported continued use of the compounds although they were aware of the threat that the compounds posed to nontarget wildlife (Morzillo and Mertig 2011a). If wildlife are especially likely to be exposed to ARs due to use of these compounds in residential areas, then measures that address residential use of ARs may be particularly effective in mitigating ecological risks associated with these compounds.

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