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Rodenticide Exposure Among Endangered Kit Foxes Relative to Habitat Use in an Urban Landscape

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Rodenticide Exposure Among Endangered Kit Foxes Relative to Habitat Use in an Urban Landscape

Endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) inhabiting Bakersfield, California exhibit a high incidence of exposure to anticoagulant rodenticides (ARs). We examined kit fox habitat use in an effort to determine potential sources of AR exposure. Kit fox capture, den, night, and mortality locations were assigned to one of 10 habitat categories. Using all available locations, foxes that tested positive for second generation anticoagulant rodenticides (SGARs) were located more frequently on golf courses while those testing negative were located more frequently in commercial areas. Foxes that tested positive for first generation anticoagulant rodenticides (FGARs) were located more frequently in industrial areas while those testing negative were located more frequently on golf courses. Based on night locations (when foxes are foraging), foxes that tested positive for SGARs were found more frequently in undeveloped and golf course habitats. Foxes that tested positive for FGARs were found more frequently in undeveloped, campus, and industrial habitats. Although available data were not sufficient to identify specific point-sources of AR exposure for foxes, golf courses appeared to be used more frequently by foxes exposed to SGARs. However, sources of exposure likely are abundant and widespread in the urban environment. Based on the results of this study, we recommend (1) investigating patterns of AR use in Bakersfield, (2) conducting an outreach program to emphasize the risk from ARs to kit foxes and other wildlife, and (3) continuing to monitor the incidence and patterns of AR exposure among kit foxes in Bakersfield.

Keywords
endangered species, habitat use, rodenticides, San Joaquin kit fox, urban environment, Vulpes macrotis mutica

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INTRODUCTION

Anticoagulant rodenticides (ARs) are used extensively to control vertebrate pest populations. These compounds act as Vitamin K agonists to interfere with blood clotting and cause mortality through internal hemorrhaging, typically after a lag phase of several days. The target pest can continue to consume bait during the lag phase, causing super-lethal concentrations to accumulate in its body. Predators and scavengers consuming the rodent are thus exposed to very high doses of these toxic compounds. Anticoagulant rodenticides can be first-generation (FGAR) or second-generation (SGAR). Although the mechanism for toxicity is the same, SGAR products are much more toxic and persistent in biological tissue, and are, therefore, only legally used to control commensal rodents. FGARs can be used to control either commensal rodents or field rodents. The threat to non-target wildlife is likely elevated in or near urban areas where use of ARs may be extensive (Stone et al. 1999, Hosea 2000, Riley et al. 2007, Bartos et al. 2012). Of particular concern are AR impacts involving non-target species that are rare or sensitive (Hosea 2000, McMillin 2008). Mortalities from ARs have been reported for a number of at-risk mesocarnivore species including fishers (*Martes pennanti*; Gabriel et al. 2012), European mink (*Mustela lutreola*; Fournier-Chambrillon et al. 2004), island foxes (*Urocyon littoralis*; J. King, Catalina Island Conservancy, personal communication; N. Gregory, Institute for Wildlife Studies, personal communication), and San Joaquin kit foxes (*Vulpes macrotis mutica*; Standley et al. 1992, Hosea 2000, McMillin et al. 2008).

The San Joaquin kit fox is a distinct subspecies endemic to arid shrubland and grassland habitats in central California. This subspecies is listed as Federal Endangered and California Threatened, primarily due to profound habitat loss and degradation throughout its range (U.S. Fish and Wildlife Service [USFWS] 1998). AR poisoning also has been identified as both a historic and current potential threat to kit foxes (USFWS 1998, 2010). Rodenticides, including anticoagulant compounds and strychnine, have been identified as the cause of mortality for a limited number of San Joaquin kit foxes (Huffman and Murphy 1992, Standley et al. 1992, Cypher 2010, California Department of Fish Wildlife unpublished data). However, such mortalities are likely under-reported because of a paucity of population monitoring efforts (particularly on private lands), the likelihood that foxes die underground in their dens, and the fact that foxes debilitated by AR poisoning may succumb to other more obvious proximate mortality sources (e.g., predators, vehicles). Thus, the true frequency of occurrence of mortalities from ARs is unknown.

Urban development is responsible for significant habitat loss in the San Joaquin Valley. Paradoxically, a population of kit foxes occurs in the city of Bakersfield. This kit fox population numbers several hundred individuals and appears to be persistent and demographically robust (Cypher 2010, Cypher et al. 2012). Primary sources of mortality include vehicle strikes and larger predators (e.g., coyotes [*Canis latrans*] and domestic dogs [*Canis familiaris*]), but some fox deaths have been attributed to toxins, particularly ARs (Bjurlin et al. 2005). McMillin et al. (2008) reported that 27 of 30 kit foxes from Bakersfield that were tested had liver residues of at least one AR, and in some cases multiple ARs were present. Both FGAR and SGAR compounds were detected, although the SGARs were detected at a much greater frequency.

The sources of ARs found in kit foxes in Bakersfield are unknown. Indeed, also unknown is whether kit foxes are ingesting ARs through primary exposures (i.e., direct consumption of rodenticides) or secondary exposures (i.e., consumption of dead or morbid animals that have ingested ARs). In 2011 and 2012, six kit foxes found dead in Bakersfield were determined to have died from strychnine poisoning (S. McMillin, California Department of Fish and Wildlife, unpublished data). Secondary toxicity from strychnine is rare and it is only legally applied underground, indicating that the fox deaths resulted from either intentional or unintentional misuse. Thus, primary exposure may also be a route for ARs.

The urban environment consists of a heterogeneous matrix of land uses. Use of rodenticides most likely varies considerably among these land uses depending upon the presence and abundance of rodents, and the degree of nuisance or damage issues associated with these rodents. In addition, pesticide product labels specify where products maybe be used and for what pest. SGARs are legally used only for commensal rodents (e.g., house mice [*Mus musculus*] and rats [*Rattus spp.*]) within 100 feet of structures. However, FGARs can be used both in the field away from structures as well as in or near structures. Use of these different habitats by kit foxes also varies depending upon ease of access, presence of food and den locations, and presence of threats. Thus, rodenticide exposure risk likely varies with land use.

The goal of this study was to attempt to identify potential sources of AR exposure for urban kit foxes based on available spatial data for individual animals. We used capture, den, night movement, and mortality locations to examine patterns of habitat use by foxes, and where possible we compared such patterns between foxes with and without exposures. Based on the results, we developed recommendations regarding possible management actions and information needs.

METHODS

Study area

The city of Bakersfield is located in the southeastern corner of the San Joaquin Valley in central California (Fig. 1). Bakersfield has a human population of over 350,000 (U.S. Census Bureau 2013), and is the largest of the 3 urban areas known to be inhabited by San Joaquin kit foxes. The city is in the southern portion of the range of the San Joaquin kit fox, and the urban environment still retains connectivity with natural habitat on the north and east sides (Fig. 1). Kit foxes are commonly observed in Bakersfield and the urban population may number several hundred individuals (Cypher 2010, Cypher and Van Horn Job 2012).

Kit foxes

Investigations of urban kit fox demography and ecology were initiated in 1997 (Cypher 2010), and testing of foxes for anticoagulant rodenticide exposure was initiated in 2000 as part of a larger investigation of exposure rates in wildlife (Hosea 2000, McMillin et al. 2008, McMillin 2012). A kit fox was included in this investigation if (1) a carcass had been recovered upon mortality, (2) a liver sample had been collected from the carcass and submitted for AR testing, (3) one or more exact locations (either capture, den, night movement, or mortality – see below)

were available for the fox, and (4) the age of the fox at death was greater than 4 months. The age restriction was implemented because foxes 4 months old or younger generally are still being provisioned by parents at natal dens, and therefore any rodenticide exposure is more likely to be a function of foraging areas selected by the parents and not habitat use by these young foxes.

Fox carcasses were recovered in several ways. Some carcasses were located as a result of various radio telemetry studies conducted on urban kit foxes. Radio collars typically were equipped with mortality signals that facilitated the timely collection of dead foxes. Other carcasses were opportunistically found by researchers or the public. In a few cases, morbid foxes were observed, captured, and taken to local veterinarians where they subsequently died or were euthanized, and then the carcasses were collected for further studies. Finally, a number of older carcasses were found stored in a freezer at the California State University-Bakersfield and made available for further studies.

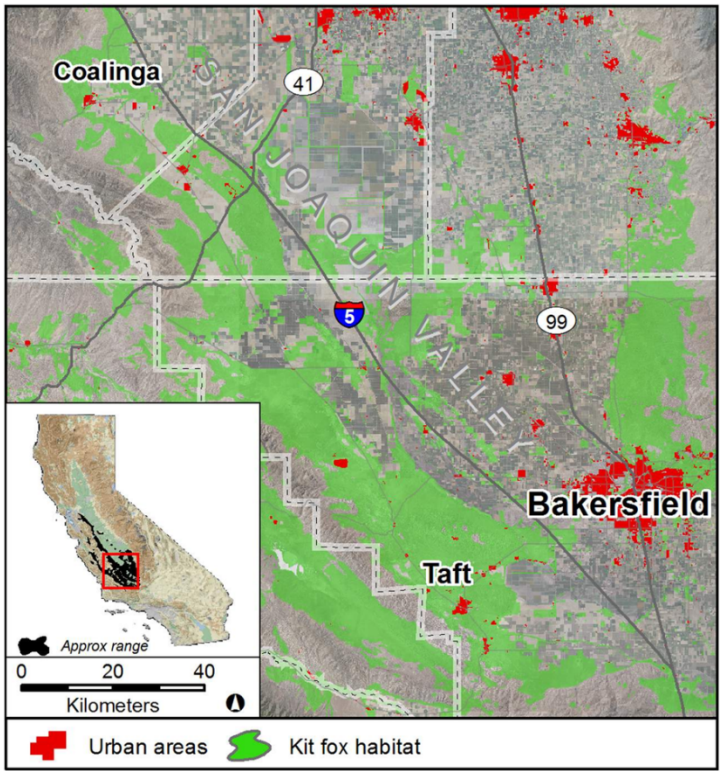

Figure 1. Urban areas with populations of San Joaquin kit foxes in the San Joaquin Valley of California

Anticoagulant rodenticide analyses

Liver samples were collected from fox carcasses, placed in labeled containers, and stored frozen until analysis. All samples were submitted to the Wildlife Investigations Laboratory of the California Department of Fish and Wildlife (CDFW) in Rancho Cordova, CA, where, in preparation for analysis, the tissues from each sample were homogenized. Analysis of the samples for the presence of ARs was conducted over a multi-year period as both samples and funding to analyze samples became available. Consequently, the analyses were conducted at three different analytical laboratories depending upon which one CDFW had contracted with in a given year. The three were: the CDFW Water Pollution Control Laboratory in Rancho Cordova, CA; the Mississippi State Chemical Laboratory in Mississippi State, MS; and the California Animal Health and Food Safety Laboratory in Davis, CA. Samples were analyzed using high-performance liquid chromatography with mass spectrometry. Detection limits for each rodenticide varied by laboratory (Table 1) due to slight differences in analytical methods. Samples analyzed in 2002 and prior had higher detection levels than those analyzed later in the study. The consequence of these higher detection limits may have been fewer detections in earlier samples. However, of the 18 samples with higher detection limits, 15 were positive for SGARs and 5 were positive for FGARs. The ARs for which samples were tested were brodifacoum, bromadiolone, difethialone, chlorophacinone,

diphacinone, coumatetralyl, warfarin, and pival. The first three are considered SGARs and the last five are considered FGARs. FGARs are less toxic and require multiple feedings by target species whereas SGARs are much more toxic with target species typically succumbing after just one feeding (Hadler and Buckle 1992).

Table 1. Anticoagulant rodenticides tested for in San Joaquin kit foxes, common commercial products containing each rodenticide, generation (1st or 2nd), and laboratory detection limits.

			Detection limits (ng/g)[1]		
Rodenticide	**Common Products**	**Gener-ation**	**WPCL[2]**	**MSCL[3]**	**CAHFSL[4]**
Brodifacoum	d-Con, Talon, Havoc, Ratak, Volak, Volid, Klerat	2nd	0.2	7.0	50.0
Bromadiolone	Apobas, Bromard, Bromatrol, Bromone, Bromorat, Candien 2000, Contrac, Contrax, Deadline, Hurex, Lanirat, LM637, Maki, Morfaron, Musal, Ramortal, Ratimon, Ratimus, Roine-C, Slaymor, Super-Caid, Sup'operats, Termus, Topidon	2nd	0.2	7.0	10.0
Difenacoum	Comp, Dephenacoum, Matrak, Neosorexa, Rastop, Ratak, Ratrick, Silo	2nd	0.2	7.0	250.0
Chlorophacinone	AFNORR, Caid, Delta, Drat, Liphadione, LM 91, Microzul, Muriol, Quick, Ramucide, Ranac, Ratomet, Raviac, Rozol, Topitox	1st	2.0	50.0	250.0
Diphacinone	Diphacin, Promar, Ramik	1st	2.0	50.0	250.0
Coumatetralyl	Racumin, Stunt, Ratryl, Cumakil	1st	1.0	50.0	50.0
Warfarin	d-Con, Rax, Cov-R-Tox, Kypgarin, Rodex, Tox-Hid	1st	1.0	50.0	50.0
Pival	Pivalyn, Pival, Pindone	1st	N/A	N/A	250

[1] ng/g = nanograms of rodenticide per gram of liver sample (= parts per billion);
[2] WPCL = CDFW Water Pollution Control Laboratory;
[3] MSCL = Mississippi State Chemical Laboratory;
[4] CHFSL = California Animal Health and Food Safety Laboratory

Kit fox locations and habitat use

Kit fox locations consisted of capture locations, den locations, night locations, and mortality locations. In conjunction with various demographic and ecological research projects conducted by the California State University-Stanislaus, Endangered Species Recovery Program, kit foxes were live-captured to collect biological data, mark individuals, and obtain genetic data, and some foxes were fitted with radio collars. Foxes were physically restrained without chemical immobilization, and then released at the capture site after processing. Most radio-collared individuals were tracked to their dens 1-4 times per week with the frequency dependent upon

specific research objectives. Kit foxes exhibit year-round diurnal den use to avoid predators, avoid temperature extremes, conserve moisture, rest, and rear young (Koopman et al. 1998, Cypher 2003). Some radio-collared kit foxes also were located visually while foraging at night. Signals for all foxes minimally were heard at least once each week which usually provided ample time to recover any dead foxes and collect samples for AR analysis before tissues became unusable. Finally, mortality locations were collected for all foxes found dead. Global Positioning System coordinates were determined for all locations. Detailed methods for trapping, collaring, and tracking kit foxes are described in Cypher et al. (2000) and Bjurlin et al. (2005). Collection of San Joaquin kit fox carcasses and liver samples and all capture and handling of foxes were conducted under U.S. Fish and Wildlife Service permit TE-825573 and a Memorandum of Understanding from the California Department of Fish and Wildlife. Capture and handling methods were consistent with the guidelines established by the American Society of Mammalogists for care and use of animals in research (Sikes et al. 2011).

Ten habitat categories were defined broadly based on common land uses and the estimated potential use of FGARs and SGARs on those lands (Table 2). Each kit fox location was assigned to a habitat category. For many locations, particularly den and night locations, a habitat description was recorded at the time the fox was located. For all other locations, coordinates were plotted on a base map in Google Earth to determine the habitat category. Google Earth was used because the base map could be adjusted to reflect habitat conditions on or near the date when the location was recorded. This was extremely helpful because the urban environment in Bakersfield is quite dynamic with land use patterns changing annually (e.g., as new urban development occurred).

Table 2. Habitat types used by San Joaquin kit foxes in Bakersfield, CA, rodent species found in each type, and potential risk of anticoagulant rodenticide exposure.

			Estimated potential for rodenticide use	
Urban habitat category	**Description**	**Potential target rodents[1]**	**1st Generation**	**2nd Generation**
Canal	Banks and right-of-ways associated with canals	Ground squirrels, gophers – control efforts commonly implemented	High	Low
Construction	Areas cleared and graded upon which buildings, parking areas, landscaping, etc., are being constructed	Few or no rodents due to disturbance – few control efforts	Low	Low
Golf course	Golf courses and associated facilities	Ground squirrels, gophers, commensal rodents - control efforts commonly implemented	High	High
Residential	Areas with single-family and multi-family dwellings	Commensal rodents - control efforts commonly implemented	Low	High
Undeveloped	Vacant lots (with or without vegetation), storm water drainage basins, city parks	Ground squirrels, gophers, commensal rodents - control efforts infrequently implemented	Low	Low
Commercial	Stores, offices, other businesses and associated facilities	Commensal rodents - control efforts commonly implemented	Low	High

Table 2, Continued.

			Estimated potential for rodenticide use	
Urban habitat category	**Description**	**Potential target rodents1**	**1st Generation**	**2nd Generation**
Industrial	Manufacturing facilities, pipe storage yards, oil tank settings, refineries, etc.	Ground squirrels, gophers, commensal rodents - control efforts commonly implemented for commensal rodents	Low	High
Campus	Schools and colleges and associated facilities	Ground squirrels, gophers, commensal rodents - control efforts commonly implemented	High	High
Linear	Power line and railroad corridors	Ground squirrels, gophers, commensal rodents - control efforts infrequently implemented	Low	Low
Agriculture	Alfalfa fields	Ground squirrels, gophers – control efforts commonly implemented	High	Low

Data analyses

The frequency of occurrence of kit fox locations among habitat types was compared between foxes with and without exposures for both FGARs and SGARs. Comparisons were conducted using contingency table analysis with a χ^2 test statistic. A Yate's correction-for-continuity value of 0.5 was used for 2x2 contingency tables (Zar 1984). Comparisons were conducted using all kit fox locations (i.e., capture, den, night, and mortality locations). Only a mortality location was available for some foxes while others had numerous locations of multiple types. To control for weighting effects and associated potential biases resulting from individual foxes being relocated repeatedly in the same location (e.g., den locations), statistical comparisons were repeated using only the mortality locations for each fox. Thus, only one location was used per fox. Finally, foxes were most likely to encounter rodenticide baits or poisoned rodents while foraging. The night locations were the ones most likely to reflect habitats used while foraging, and hence, the potential locations of exposure sources. Therefore, additional analyses were conducted using only night locations. Unfortunately, all of the foxes with night locations also had been exposed to SGARs, and thus, habitat use patterns could not be compared to non-exposed foxes. Habitat use by these animals was examined using a goodness-of-fit χ^2 test with a null hypothesis of equal proportions of locations in all habitat types. For all analyses, p-values ≤ 0.05 were considered statistically significant.

RESULTS

Anticoagulant rodenticide exposure

A total of 68 kit foxes met the criteria for inclusion in this study. Collection dates for the carcasses ranged from 1985 to 2009, although most were collected during 1998 to 2009 when more intensive field research efforts were being conducted on the Bakersfield kit fox population.

Because testing was conducted over a number of years and involved multiple laboratories, not all samples were tested for all ARs. Of the 68 foxes, AR residues were detected in 50 (73.5%) and two or more rodenticides were detected in 29 (42.6%). Brodifacoum and bromadiolone were the most commonly detected ARs and were found in 69.1% and 38.2%, respectively, of foxes tested (Table 3). Chlorophacinone, coumatetralyl, and pival also were detected but only infrequently, while difenacoum, diphacinone, and warfarin were not detected in any foxes tested. Overall, residues of SGARs were detected in 50 of 68 (73.5%) foxes tested while FGARs were detected in 8 of 60 (13.3%). All foxes with FGARs also tested positive for SGARs. Detection limits for each rodenticide varied by laboratory with samples analyzed prior to 2003 having higher detection limits than those analyzed later in the study. The consequence of these higher detection limits may be that there were fewer detections among earlier samples. However, of the 18 samples analyzed with higher detection limits, 15 were positive for SGAR anticoagulants and 5 were positive for FGARs, indicating that anticoagulants were being detected at frequencies comparable to those for samples subsequently analyzed with lower limits.

Table 3. Number of kit foxes tested, number of detections and the range of residue concentrations for 8 anticoagulant rodenticides.

Rodenticide	Number of foxes tested	Number of detections	Proportion exposed (%)	Residue concentration range (ng/g)
Brodifacoum	68	47	69	0.20 - 11,000
Bromadiolone	68	26	38	1.17 - 3,132
Difenacoum	14	0	0	-
Diphacinone	60	0	0	-
Chlorophacinone	60	4	7	49.2 - 270
Warfarin	47	0	0	-
Coumatetralyl	43	5	12	134 - 1420
Pival	14	1	7	6,930 (1 detection)

Kit fox locations

In total, 2,254 locations and associated habitat classifications were available for the 68 foxes included in this study. When all locations were considered, there were 2,229 for the 50 foxes that tested positive for SGARs and just 25 locations for the 18 foxes that tested negative. All foxes were located most frequently in undeveloped habitats (Fig. 2a). However, proportional use of habitat types differed ($\chi^2 = 66.7$, 9 df, $p < 0.001$) with exposure. Foxes that tested positive for SGARs were located more frequently on golf courses while those testing negative were located more frequently in commercial areas. Use of other habitats was generally similar.

There were 809 locations for the eight foxes that tested positive for FGARs and 1,411 locations for the 52 foxes that tested negative. All foxes were located most frequently in undeveloped habitats (Fig. 2b). However, proportional use of habitat types differed ($\chi^2 = 603.3$, 9 df, $p < 0.001$) with exposure. Foxes that tested positive for FGARs were located more frequently in industrial areas while those testing negative were located more frequently on golf courses. Use of other habitats was generally similar.

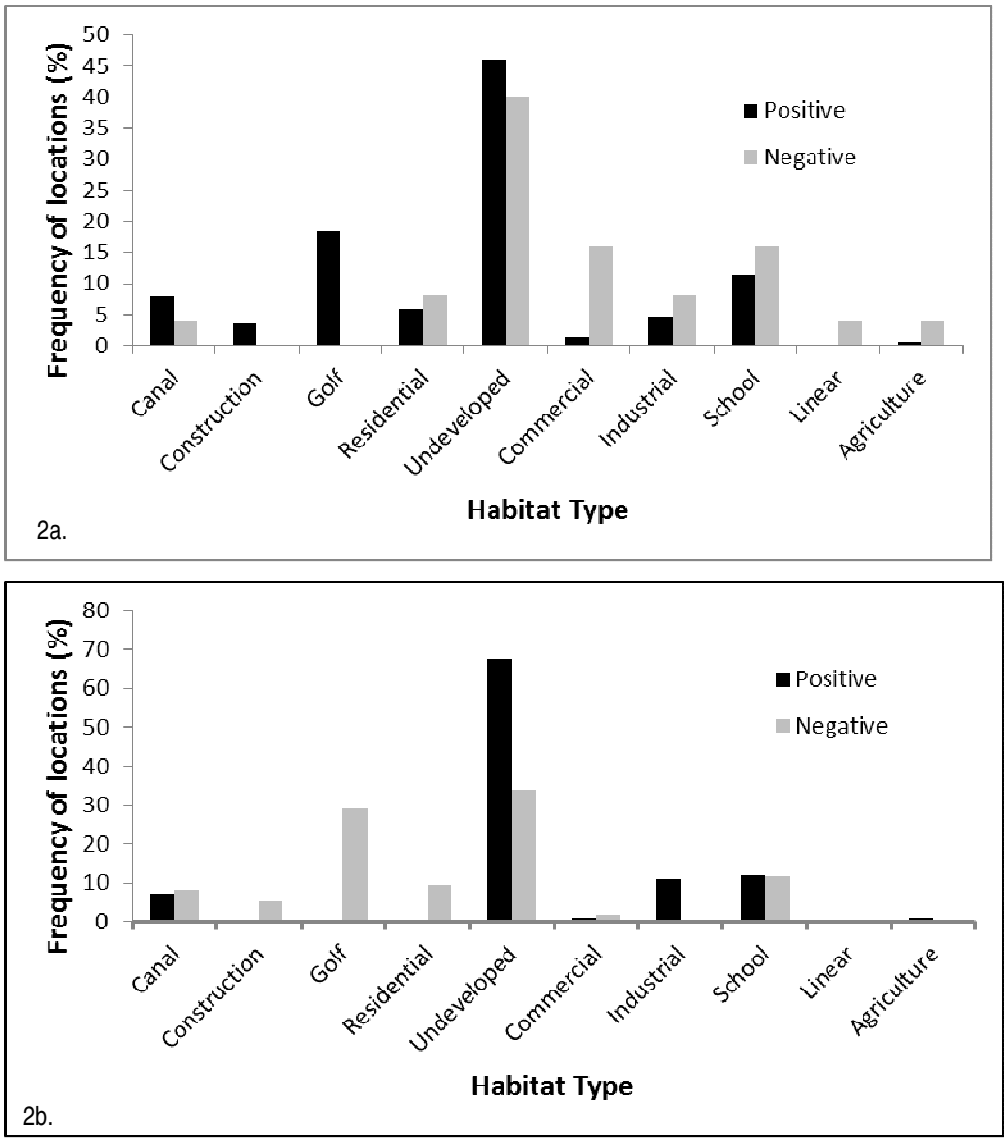

Figure 2. Proportional use of habitats by San Joaquin kit foxes that tested positive or negative for (a.) second generation or (b.) first generation anticoagulant rodenticides, Bakersfield, CA, based on all locations (i.e., capture, den, night, and mortality).

Using just mortality locations reduced the sample sizes to one location for each of the 50 foxes that tested positive for SGARs and each of the 18 foxes that tested negative. Among all foxes, carcasses were found most frequently in undeveloped, commercial, and campus habitats (Fig. 3a), and proportional distribution among habitat types did not differ ($\chi^2 = 7.15$, 9 df, $p = 0.62$) with exposure. For the eight foxes that tested positive for FGARs and 52 foxes that tested negative, carcasses were found most frequently in undeveloped habitats (Fig. 3b). Proportional distribution among habitat types did not differ ($\chi^2 = 4.63$, 9 df, $p = 0.87$) with exposure.

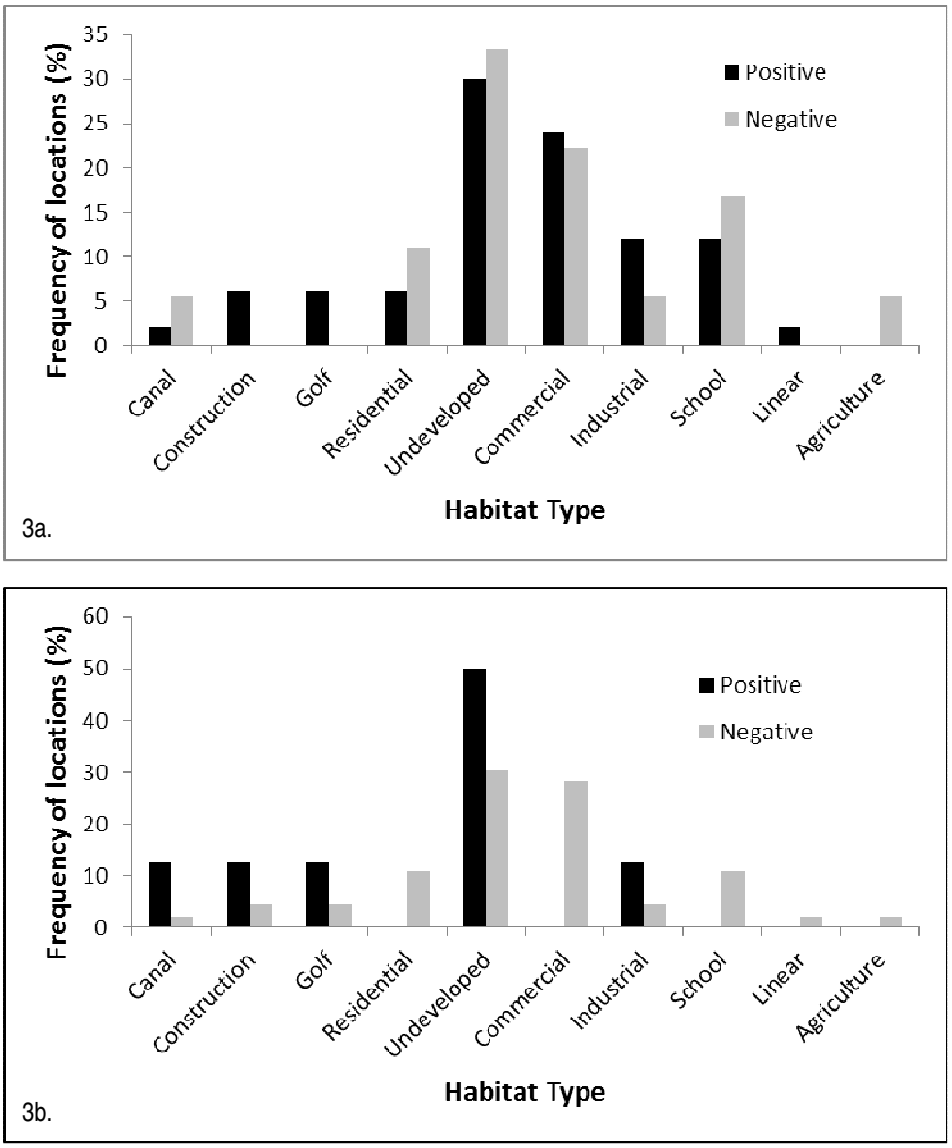

Figure 3. Proportional use of habitats by San Joaquin kit foxes that tested positive or negative for (a.) second generation or (b.) first generation anticoagulant rodenticides, Bakersfield, CA, based on mortality locations.

Just using night locations for kit foxes, there were 1,000 for the 15 foxes that tested positive for SGARs. No night locations were available for foxes that tested negative. For the foxes testing positive, the majority of the locations were in undeveloped and golf course habitats (Fig. 4a). There were 211 night locations for the three foxes that tested positive for FGARs and 787 locations for the 11 foxes that tested negative. Proportional use of habitat types (Fig. 4b) differed with exposure ($\chi^2 = 310.7$, 7 df, $p < 0.001$). Foxes that tested positive for FGARs were located more frequently in campus and industrial habitats while those testing negative were located more frequently in golf course and residential habitats.

Some foxes had particularly high levels of ARs in their livers. Four foxes had brodifacoum concentrations exceeding 5,000 ng/g. An adult female (at least 5 years old) had a brodifacoum concentration of 5,662 ng/g and most of her locations (177 out of 192) were in undeveloped areas, particularly undeveloped lots and storm water drainage basins. The other three foxes had even higher concentrations and these foxes appeared to have a strong association with golf courses. A juvenile male (9.5 months old) had a brodifacoum concentration of 8,648 ng/g and 75 of his 79 locations were on a golf course. An adult female (at least 24.0 months old) had a brodifacoum concentration of 9,855 ng/g and 86 of her 92 locations were on a golf course. Finally, a juvenile female (only 6.0 months old) had a brodifacoum concentration of 11,000 ng/g, and indeed, AR poisoning was determined to be the cause of death for this animal. Only 2 locations were available for this fox, but one was on a golf course and the other (mortality location) was at an office complex adjacent to the golf course. An adult female (13.5 months old) had a pival concentration of 6,930 ng/g. This fox routinely used several habitats including a water tank setting, a storm water drainage basin, and a canal.

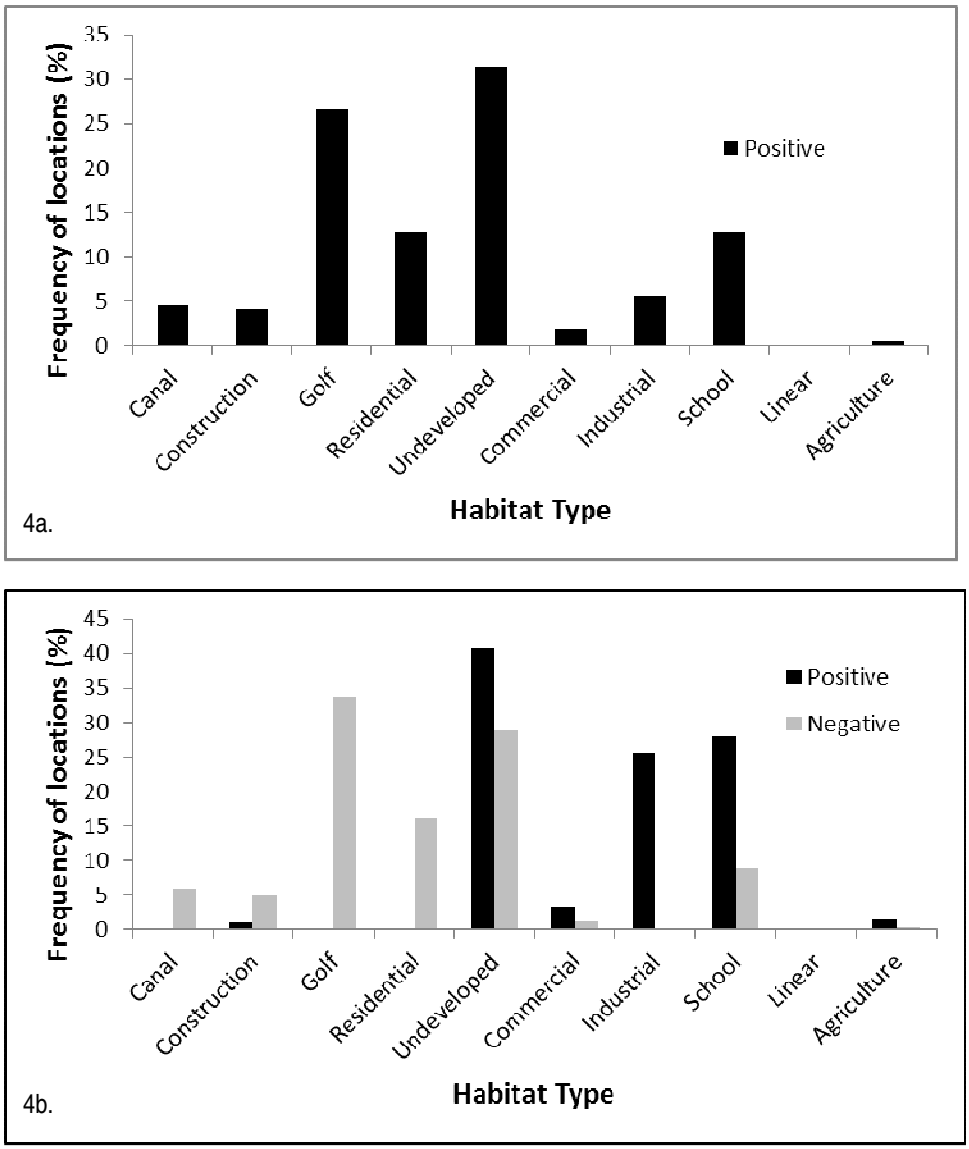

Figure 4. Proportional use of habitats by San Joaquin kit foxes that tested positive for (a.) second generation or (b.) first generation anticoagulant rodenticides, Bakersfield, CA, based on night locations

DISCUSSION

Use of ARs in urban environments appears to be routine and extensive (Riley et al. 2007, Morzillo and Schwartz 2011, Bartos et al. 2012), and the results of this study were consistent with that observation. We documented a high incidence of exposure among foxes collected over many years throughout Bakersfield, particularly for SGARs. Brodifacoum and bromadiolone were frequently detected in liver samples from San Joaquin kit foxes in Bakersfield. These two SGAR compounds commonly are the active ingredients in over-the-counter products (see Table 1) used to control commensal rodents. In fact, 89% of brodifacoum is used by non-licensed people such as homeowners and maintenance workers (California Department of Pesticide Regulation 2012). Bromadiolone products are more commonly used by professional applicators to treat structures. Thus, use is likely prevalent and wide-spread, and in that sense their presence in kit foxes is not surprising.

FGAR compounds were detected relatively infrequently. Chlorophacinone was detected in just four animals. This FGAR is most commonly used in grain-based baits that target ground squirrels. Squirrel control efforts are less common in urban environments, and even when conducted, use of grain-based baits may be infrequent due to the potential for exposure by song birds, domestic animals, and people. For example, on the California State University-Bakersfield campus, grain-based rodenticides were first replaced with gas-based (e.g., aluminum phosphide) methods and more recently with live-trapping, specifically to avoid harm to kit foxes (W. Laurendine, Live Oak Associates, pers. comm.). Also, FGAR compounds are less persistent in tissues, which may inhibit detection of exposures. For example, warfarin has a half-life of between 5 and 28 hours in tissues (Hadler and Buckle 1992). The presence of coumatetralyl and pival in foxes was surprising. Coumatetralyl is not registered for use in the United States, and the registration for pival was suspended in 1994 (U.S. Environmental Protection Agency 1998).

Whether AR exposures by kit foxes are primary (i.e., direct consumption of rodenticide-laced baits) or secondary (i.e., consumption of prey items contaminated with rodenticides) is unknown. Some SGAR baits are presented in trays or other open containers, and some FGAR baits are dispersed on open ground or entrances of rodent burrows (B. Cypher, personal observation) where they are accessible to kit foxes. In addition, flavorizers added to baits to increase attractiveness to rodents may also increase attractiveness to foxes. While SGAR compounds are only legally used for commensal rodents, they may be used outside as long as they are within 100 feet of a man-made structure. FGAR compounds can be used for commensal rodents or field rodents, which means they can be found in and around structures, as well as independent of structures. In all of these cases, foxes potentially could access and consume baits resulting in a primary exposure. Also, disturbingly, in a mail survey conducted among Bakersfield residents, some number of the 317 respondents stated that they had used rodenticides specifically in an attempt to “control” kit foxes (Morzillo and Schwartz 2011).

The potential for secondary exposure also is high. Secondary exposure was suspected as the source of SGAR residues in livers of mountain lions (*Puma concolor*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), and various raptors (Riley et al. 2007, Lima and Salmon 2010, Moriarty et al. 2013). Foxes readily consume rodents in the urban environment, particularly gophers and ground squirrels (Cypher 2010). Thus, exposure could occur through consumption of prey items,

particularly dead or morbid rodents. Urban foxes also consume birds and insects, which present another potential source of secondary exposure. Birds can consume baits or contaminated prey, and then be consumed by foxes. Godfrey (1985) reported that birds in a zoo aviary died after consuming insects that had fed on bait containing brodifacoum. Finally, young foxes could be exposed to ARs through provisioning of contaminated food items by parents. ARs also can be passed to embryos through trans-placental transmission (Munday and Thompson 2003) and possibly to nursing neonates through lactational transfer (Gabriel et al. 2012). Thus, urban kit foxes could be exposed to ARs via a number of pathways.

Anticoagulant rodenticide exposure relative to habitat use

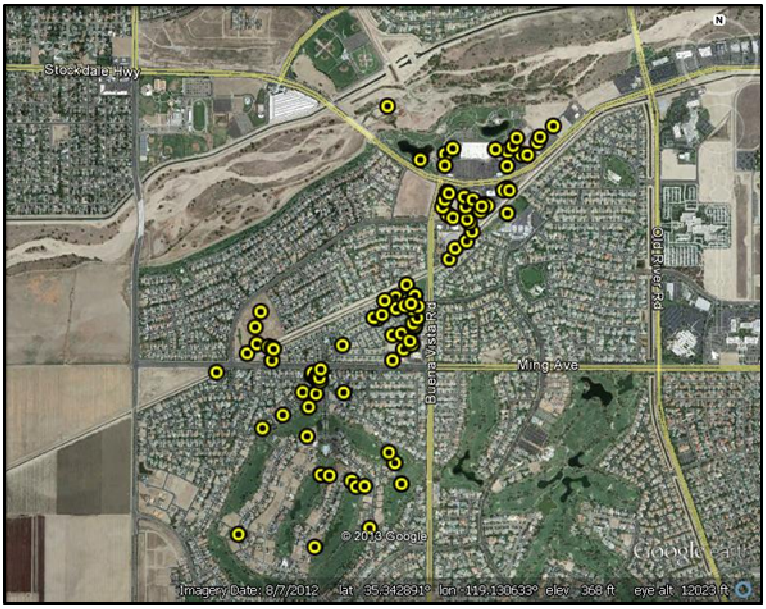

Figure 5. Night locations for an adult female San Joaquin kit fox in Bakersfield, California that regularly used multiple habitats including Canal (38 locations), Construction (17 locations), Golf Course (54 locations), Residential (82 locations), and Undeveloped (21 locations).

Identifying specific habitats that might constitute a higher risk of AR exposure for foxes was challenging. Part of this challenge stems from the high habitat heterogeneity in urban landscapes. Many of the kit foxes inhabiting Bakersfield routinely use multiple habitat types (Fig. 5), including on a nightly basis. Furthermore, our data set was not ideal in that the number of locations for individual foxes ranged from 1 to 334, and therefore the results were biased by animals for which we had more data. However, our analyses did reveal some trends that may indicate habitats with higher potential for exposure. One such trend is that golf courses appeared to be used more frequently by foxes that had been exposed to SGARs. Some urban kit foxes use golf courses extensively (Fig. 6, 7). For many reasons, including aesthetics, functionality, and human safety, rodent control on golf courses may be aggressive. The same may hold true for residences surrounding golf courses. It is unknown whether the SGAR exposure in foxes is due to legal use for commensal rodent control or misuse for field rodent control. Products with SGARs also are routinely used for the control of rats and mice in residential areas (Morzillo and Schwartz 2011), and rodenticide bait stations are commonly observed in Bakersfield in commercial and campus areas.

Undeveloped areas, school campuses, and industrial areas appeared to be used somewhat more frequently by foxes that had been exposed to FGARs. The most likely targets of FGAR use in Bakersfield are ground squirrels and gophers, and both of these species can be abundant in the three habitat types mentioned above. Products containing difethialone and diphacinone are routinely used on all high school campuses in the city on an “as-needed” basis (M. Perez, Kern

High School District, personal communication). Based on anecdotal information, gopher and ground squirrel control also are routinely conducted in canal, golf course, and agricultural areas.

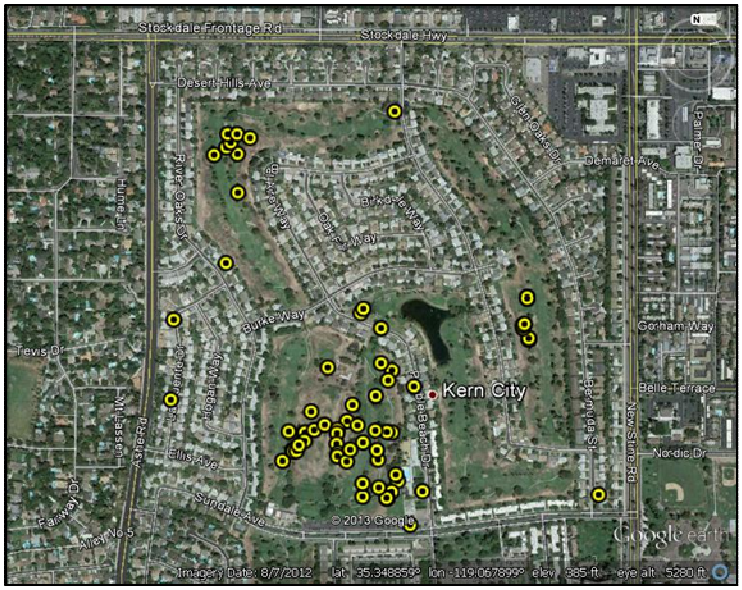

Figure 7. Night locations for an adult female San Joaquin kit fox in Bakersfield, California that primarily used Golf Course (63 locations) and occasionally Residential (5 locations) habitats.

Figure 6. San Joaquin kit fox family group on the Seven Oaks Country Club in Bakersfield, California

Of interest were foxes that had particularly high levels of ARs in their livers. It is unknown whether these high concentrations are a result of multiple exposures or one high-dose exposure. The highest level of brodifacoum was detected in a fox that was only 6 months old. This suggested that the exposure may have been the result of one or a few high-dose exposures versus many cumulative exposures over time. Most interestingly, all three of the foxes with the highest brodifacoum concentrations used the same golf course. This potentially indicates that improper practices (e.g., improper storage) might have been occurring in this area. The fox with pival in its liver used an area where ground squirrels were common and these potentially were the target of control efforts. As discussed previously, the registration for pival has been suspended since 1994. However, this fox was probably born in 2001 and eventually died in 2002. Products containing this compound that were acquired prior to the suspension apparently continued to be used or stored. It is also possible that this fox may have been exposed due to improper disposal of an old product (McMillin et al. 2008).

Coumatetralyl was found in liver samples from five foxes even though this rodenticide is not registered for use in the United States. Three of these foxes commonly used two different canals in Bakersfield. Coumatetralyl is a FGAR and likely would have been used to control ground squirrels and gophers, both of which are common target pest species along canals. Interestingly, one of these foxes also had a number of locations in the same area as the fox that was exposed to pival. Another fox that was exposed to coumatetralyl primarily was located at an elementary school and adjacent storm water drainage basin. Only one location was available for

the fifth fox, which was found dead far from the other foxes on a road in an area with primarily undeveloped lands. If the spatial data for these foxes indeed encompass sources of rodenticide exposure, then the number of apparently unrelated locations where an unregistered product was being used is cause for concern.

Potential population effects

Quantifying the impacts of AR exposure to kit foxes in urban environments is difficult. Determining whether a fox died from AR poisoning is not always straightforward. The liver concentration levels considered to be fatal to foxes are unknown. Also unknown are the effects of interactive or cumulative effects among different ARs, or the effects of fox sex, age, health status, or reproductive status. Brodifacoum toxicosis has been identified in neonatal domestic dogs (Munday and Thompson 2003) suggesting that ARs could reduce reproductive success in canids, at lower doses than would cause adult mortality. Finally, another unknown is whether ARs might induce morbidity that increases vulnerability to another proximate mortality cause, such as predation or vehicle strike. Animals exposed to anticoagulant rodenticides have been reported to display behavioral changes such as lethargy and slower reaction time (Cox and Smith 1992). Riley et al. (2007) reported that incidents of infection and mortality from notoedric mange among bobcats appeared to be associated with AR exposure. Interestingly, although sarcoptic mange has not been reported among San Joaquin kit foxes, an outbreak was detected among urban kit foxes in 2012 and at least 5 foxes are known to have died from the disease (Cypher, unpublished data). Whether there is any relationship between the mange outbreak and the high incidence of AR exposure among urban kit foxes has yet to be determined.

A determination of death from ARs commonly is based on a post-mortem examination (e.g., internal hemorrhaging), absence of other obvious mortality factors (e.g., predation or vehicle strike), chemical analysis of a liver sample, or a combination of these, but the results can be difficult to interpret. The fox with the brodifacoum liver concentrations of 11,000 ng/g did not exhibit any evidence of mortality by another source, and a necropsy revealed significant quantities of blood in the body cavity with minimal clotting. Thus, this fox exhibited strong evidence of death from AR poisoning. However, of the three foxes with the next highest brodifacoum levels, one (9,855 ng/g) was killed by a predator, another (8,648 ng/g) was killed by a vehicle, and the last (5,662 ng/g) appeared to have a non-fatal puncture wound and cause of death could not be determined. Conversely, AR poisoning was identified as the putative cause of death for three other foxes with pooled, unclotted blood in their body cavity, but subsequent analysis of liver samples revealed lower brodifacoum levels than had been present in asymptomatic animals: 1,037 ng/g (plus 17.2 ng/g of bromadiolone), 186 ng/g (plus 24.0 ng/g of bromadiolone and 49.2 ng/g of chlorophacinone), and 7.0 ng/g (plus 7.0 ng/g of bromadiolone) for these three animals.

With the many unknowns identified above, the impacts and risk of ARs to the urban kit fox population in Bakersfield cannot be precisely quantified. Kit foxes appear to be quite abundant in Bakersfield and are demographically robust (Cypher 2010). However, the high proportion of animals that have been exposed to ARs and strong evidence that some foxes have died from AR poisoning are cause for concern. The urban kit fox population in Bakersfield is considered important for conservation and recovery of the species (Cypher 2010, Cypher and

Van Horn Job 2012). Small kit fox populations also occur in Taft and Coalinga (see Fig. 1), and additional populations could become established over time in other urban areas. All such populations will increase in importance as natural habitat continues to decline in the San Joaquin Valley. Thus, any measures that reduce kit fox exposure to and risks from ARs will be beneficial. In May 2008, the U.S. Environmental Protection Agency prohibited the over-the-counter sale of SGARs beginning in 2011(Bradbury 2008), and in February 2013 issued a notice of intent to cancel registration for a number of products containing both SGARs and FGARs that did not comply with the new regulations (Federal Register 2013). However, D-Con products still remain available for sale to the public pending an appeal of the cancellation order. Additionally, in 2013 at the request of the California Department of Fish and Wildlife, the California Department of Pesticide Regulation introduced a regulation that would designate all SGARs as California restricted materials, meaning only certified applicators would be able to use them. This regulation is expected to take effect in 2014. Continued monitoring will be necessary to determine whether this new regulation is successful in reducing exposure levels and poisoning cases among urban kit foxes.

Recommendations

Identify patterns of rodenticide use.—To more effectively address AR exposure issues in urban areas, information is needed regarding use patterns for ARs. Additional studies should focus on identifying problematic use practices and specific hot spots where exposure is most likely to occur. One potential source of use information is the California Department of Pesticide Regulation, which tracks sales of pesticides in California. Additionally, all agricultural uses of pesticides are reported by county, commodity, pounds applied, and acres treated. However, these data may not be of sufficient spatial resolution to identify locations where foxes might be exposed. Another challenge is that most brodifacoum, the rodenticide that is most frequently found in kit foxes, is used by non-licensed individuals such as homeowners and maintenance workers (California Department of Pesticide Regulation 2012) who are not required to report usage. If the SGARs become California-restricted use in 2014 as expected, most usage of these materials will be reported to the California Department of Pesticide Regulation. However, it will also be helpful to include other approaches such as surveys (via mail) or interviews to obtain a more accurate assessment of rodenticide use patterns.

Conduct outreach program.—An outreach program could be conducted in an effort to further inform the public about proper use of rodenticides and risks to natural resources from improper and even proper use of these substances. This program should especially target groups that likely use rodenticides frequently and in quantity over large areas, such as school campus groundskeepers, canal operators, golf course grounds maintenance staff, and pest control applicators. However, information should also be made available to the general public, both because it might produce a surveillance effect and because some members of the public likely use over-the-counter rodenticide products.

Continue monitoring exposure levels in kit foxes.—Rodenticide exposure among kit foxes should continue to be monitored to determine if new state and federal regulations or outreach efforts are effective in reducing exposure levels. A more systematic sampling strategy, as opposed to the opportunistic one employed in this study, would provide better information on the

proportion of the kit fox population exposed to ARs as well as spatial and temporal patterns of exposure.

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