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Detection of Rodenticides in Pregnant Mountain Lions (*Puma concolor*) and Their Fetuses in California

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Abstract

Anticoagulant rodenticides (ARs) are used worldwide to control rodent pests. There are 2 general groups of ARs: first-generation anticoagulant rodenticides (FGARs) and second-generation anticoagulant rodenticides (SGARs). Both pose a significant risk of toxicosis to non-target wildlife. In 2014, the California Department of Pesticide Regulation adopted regulations restricting the use of SGARs by the public to reduce non-target exposure. Despite these restrictions, SGAR exposure in wildlife, including mountain lions (*Puma concolor*), remained higher than FGAR exposure. Consequently, in 2021, legislation prohibiting the public and commercial use of SGARs in California was enacted. Here, we document exposure to 2 or more ARs in 8 pregnant mountain lions necropsied between 2016 and 2023. Nine full-term fetuses from 3 of the pregnant mountain lions had exposure to ≥1 AR, demonstrating that ARs can cross the placenta. We also document diphacinone exposure in placental tissue from 1 pregnant mountain lion. Additionally, 1 pregnant female had exposure to the neurotoxic rodenticide, bromethalin. None of the females nor their fetuses had evidence of toxicosis or obvious birth deformities. However, ARs can cause other substantial sub-lethal impacts that could impact fitness and survival of kittens which is especially concerning for isolated mountain lion populations already experiencing reduced genetic fitness. Continued surveillance to assess efficacy of

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regulation and legislative changes, including exposure to other rodenticides such as bromethalin, and further monitoring of isolated populations to understand possible additive sub-lethal effects on reproduction are warranted.

Key Words: Bromethalin, Desmethylbromethalin, First Generation Anticoagulant Rodenticides, Mountain Lion, Placenta, Second Generation Anticoagulant Rodenticides, Toxicology, Wildlife health.

Introduction

Anticoagulant rodenticides (ARs) are widely used to control rodent populations (Jacob and Buckle 2018). Since ARs are vitamin K antagonists, toxic doses can lead to impaired blood clotting resulting in fatal coagulopathy and hemorrhaging which may occur spontaneously or initiated by traumatic injury (Rattner and Mastrota 2018). There are 2 general groups of ARs: 1) first-generation anticoagulant rodenticides (FGAR) such as warfarin, diphacinone, and chlorophacinone, and 2) second-generation anticoagulant rodenticides (SGAR) including bromadiolone, brodifacoum, difenacoum, and difethialone (Jacob and Buckle 2018). Second-generation ARs, designed to overcome the development of FGAR resistance in rodents, are more potent, require fewer feedings to achieve toxic doses, and are retained in liver tissue for longer periods of time (Fisher *et al.* 2003; Berny *et al.* 2018). Risk of toxicosis due to lethal primary or secondary exposure to FGARs and SGARs in non-target predatory and scavenging species has been well documented in a wide variety of taxa across the globe (López-Perea and Mateo 2018).

In response to widespread exposure and mortality in non-target wildlife, efforts have been made at all local, regional, state, and national levels to limit the use of ARs and potentially reduce exposures. For example, the California Department of Pesticide Regulation (DPR) adopted new regulations in 2014 restricting the sales and use of the SGARs in response to growing concerns about these risks (Quinn *et al.* 2019). However, a 2016 mountain lion (*Puma concolor*) health study evaluating the efficacy of the 2014 regulations by the California Department of Fish and Wildlife (CDFW) found that SGAR exposure remained higher than FGARs (Rudd *et al.* 2018). Later, in January 2021, Assembly Bill 1788 (AB1788, the California Ecosystems Protection Act) was passed, which prohibited the public and commercial use of the SGARs, with some exceptions by special permit. Despite these regulatory and legislative changes, the CDFW and National Park Service (NPS) continued to document SGAR exposure in non-target wildlife, including mountain lions (CDFW 2021, 2022, 2023, NPS 2023, NPS unpublished data).

Another rodenticide widely available in most major retailers is a single-feed neurotoxic rodenticide, bromethalin (McMillin *et al.* 2016). When ingested, it rapidly converts into the more toxic metabolite desmethylbromethalin (DMB), which causes neurologic dysfunction and is often fatal. Unlike the ARs which can bioaccumulate and cause primary or secondary toxicosis, bromethalin and DMB are not believed to have secondary effects after ingestion of contaminated prey; however, there are few experimental studies that support or challenge this assumption (Mastrota and Wolf 2016). Similar to ARs, bromethalin has been detected in non-target wildlife, including mountain lions, other mammalian carnivores, and raptors (McMillin *et al.* 2016, CDFW 2021, 2022, 2023; Cox *et al.* 2022; Murray and Cox 2023a, 2023b; J. Sikich and S. Riley NPS 2024, personal communication).

Less documented, however, are the lethal and sub-lethal impacts of ARs or bromethalin exposure on the dependent young or fetuses of wild animals. In humans, exposure to warfarin resulted in spontaneous abortions, central nervous system abnormalities, fatal hemorrhaging, and still births (Raivio *et al.* 1977; Hou 2004; Sathienkijkanchai and Wasant 2005; Mehndiratta *et al.* 2010; Starling *et al.* 2012; Yaqoob and Rubinstein 2019). Other abnormalities included stunted growth, low birth weight, and other congenital malformations. Similar pathologies have been observed in warfarin-exposed pregnant rats (*Rattus norvegicus*), including fetal hemorrhage and edema, stunted fetal and neonatal growth, and skeletal malformities (Morgan 2006; Chetot et al 2020a). Stillbirth or neonate death shortly after parturition with fatal hemorrhaging have also been observed in pregnant domestic dogs (*Canis faniliaris*) (Fitzek and Gembardt 1977; Munday and Thompson 2003; Fitzgerald *et al.* 2018) and an Arabian horse (*Equus ferus caballus*) (Zakian et al 2019) with a history of experimental or accidental AR exposure. In wildlife, bobcat (*Lynx rufus*) fetuses (Serieys *et al.* 2015) and nursing fishers (*Pekania pennanti*) (Gabriel et al 2012) were found to have exposure to FGARs and SGARs, although no abnormalities were noted during the necropsy or on histopathology.

The aims of this study were to determine ARs presence in fetal or placental tissues from AR-exposed pregnant mountain lions and to examine fetal exposure in relation to that of the mother. We also tested for bromethalin exposure by analysis for its toxic metabolite, DMB, in a smaller subset of maternal and fetal tissue.

Methods

The CDFW is mandated by the Fish and Game Code §§ 4807(b) to perform necropsies on all depredation mountain lion carcasses in the state. Additionally, the CDFW responds to other incidents resulting in the recovery of carcasses such as fatal traumatic injury from vehicle collisions, most commonly, or poaching, public safety, found dead, or humane euthanasia due to illness.

Similarly, the NPS has been conducting mountain lion research since 2002 in Santa Monica Mountains National Recreation Area (SMMNRA) and surrounding areas, including investigating causes of mortality. Mountain lions are handled as described in Riley *et al.* (2021). Scientific collecting permits were authorized by the California Department of Fish and Wildlife (SC-005636) and the NPS Institutional Animal Care and Use Committee (PWR SAMO Riley MtLion 2014.A3). Adult and subadult mountain lions are captured and fitted with global positioning system (GPS) radio-collars (Vectronic Aerospace, GPS Plus model, Berlin, Germany) equipped with a VHF beacon and mortality sensor that alerts the researcher after 12 h of no movement.

A total of 8 pregnant adult mountain lion cadavers were included in this study. Seven unmarked pregnant adult mountain lion cadavers were necropsied at the CDFW between January 2016 and December 2023. One collared pregnant mountain lion was recovered by NPS from the road in June 2022 after researchers were notified by the public after she was struck by a vehicle during the morning rush hour in Malibu, California. Her cadaver was necropsied at the California Animal Health and Food Safety (CAHFS) Laboratory branch in San Bernardino. Carcasses were frozen in -20°C freezers until they could be necropsied. Age, body condition, and stage of prenatal development were determined at the time of necropsy. Age was determined based on dentition, coat pattern and coloration, and body mass (Ashman *et al.* 1983; McKinney 1996; Laundré *et al.* 2000). For "Female 8," her exact age was known because she was initially examined and marked as a 1-month-old kitten in February 2017. Body condition was assessed on a 1–5 scoring system (body condition score, BCS): 1 (emaciated), 2 (thin), 3 (average/normal), 4 (heavy), and 5 (obese). Adult females with a BCS of 3 or higher were considered good. Stage of fetal maturation was estimated on length and appearance. Fetal length was measured from the crown to the base of the tail (crown-rump length). The gestation period for mountain lions is approximately 93 d (Beier *et al.* 1995). However, there is no literature detailing the various stages of prenatal development for this species or other wild felids. Therefore, we inferred prenatal stages of mountain lion fetuses from domestic cats (*Felis catus*), which have a gestation of 58–67 d (Knospe 2002).

Viscera were carefully examined both grossly and histologically for evidence of coagulopathy and hemorrhaging not associated with trauma. Samples for histopathology were collected into 10% buffered formalin and submitted to CAHFS Laboratory branch in Davis, or the CAHFS Lab in San Bernardino, California. Representative samples for formalin-fixed tissues were trimmed, embedded in paraffin, thinly sectioned, and stained with hematoxylin and eosin.

Depending on the stage of fetal development − and thus individual liver weight − fetal livers and placentas were either submitted individually (individual liver or placental weight ≥ 100 g) or pooled for AR testing at the CAHFS Lab in Davis, California. All livers and placenta were collected and archived in -20°C freezers post-necropsy until submitted for testing. Tissue samples were analyzed for 8 ARs, specifically: warfarin, chlorophacinone, coumachlor (which is not registered for use in the United States), diphacinone, bromadiolone, brodifacoum, difethialone, and difenacoum. Analyses are based on a previously published method (Smith *et al.* 2017). Briefly, a 1 g sample of tissue was fortified with stable isotope labeled internal standard (d4-diphacinone), and then homogenized and extracted into 6 mL of 10% (v/v) methanol in acetonitrile. The extract supernatant was further purified using dispersive solid phase extraction, and the resulting supernatant from this was evaporated to dryness under nitrogen. The extract was reconstituted in 1 mL of methanol and analyzed by LC-MS/MS (Agilent 1290 HPLC with Agilent 6460 Triple Quadrupole Mass Spectrometer). Identification was based on retention time match as well as presence of quantifier and qualifier ions with ion ratios matching those of reference standards run. Quantitation was done using a matrix matched reference standard curve. The limit of quantitation was 50 ppb for each analyte. Analytes were reported as trace if below the 50-ppb level but still detectable. Anticoagulant rodenticide toxicosis was determined to be a cause of death or morbidity, if 1 or more ARs were detected in the liver and coagulopathy was present with no other cause (e.g., trauma, disease) identified.

Desmethylbromethalin was used as a biomarker for bromethalin exposure in tissues. Adipose tissue or brain were tested for DMB using reverse phase ultrahigh performance liquid chromatography-mass spectrometry (Filigenzi *et al.* 2015). Briefly, a 0.5 g sample of brain or adipose tissue was extracted with 10 mL of ethyl acetate on a Geno/Grinder tissue homogenizer. The supernatant was obtained after centrifugation and the solvent was evaporated under nitrogen. While still warm, 5 mL of acetonitrile were

added, and additional sample clean-up was done using a QuEChERS dSPE EMR-Lipid tube, followed by an EMR Polish tube. The resulting extract was evaporated under nitrogen and then redissolved in 0.25 mL of methanol. It was then filtered and analyzed by LC-MS/MS using an Agilent 1290 HPLC paired with a SciEx 6500+ QTrap mass spectrometer, using electrospray positive ionization in MRM mode monitoring the 562 m/z -> 254, 278 m/z transitions. Identification was made by comparison to a reference DMB standard, based on retention time, presence of both ions, and the ion ratio of the 2 fragment ions matching within 25% of the standard. The reporting limit was 1 ppb. Positive samples below the reporting limit by LC-MS/MS were defined as trace.

Collected data was archived in Excel (Microsoft, Redmond, WA). Data visualization was performed in ArcGIS version 10.6 (ESRI, 2018, Redlands, California).

Results

The 8 pregnant mountain lions necropsied originated from 7 different California counties: Amador, El Dorado, Los Angeles, Santa Barbara, Trinity, Tuolumne, and Yuba (Figure 1). The estimated age of 7 females ranged from 3–10 yrs old. One adult female, "Female 8" was known to be 5 yrs old. The number of fetuses each pregnant individual carried varied from 2–4 (Table 1). Depredation permit mortalities accounted for 6 mountain lions, while vehicle strike accounted for 2 of them.

All 8 pregnant mountain lions had 2 or more ARs detected in their livers (Table 1). Among SGARs, brodifacoum and bromadiolone were detected in all 8 pregnant females, while difethialone was detected in 3 pregnant females. Among FGARs, diphacinone was detected in 7 pregnant females while chlorophacinone was detected in 4 individuals. Warfarin, coumachlor, and difenacoum were not detected.

In total, there were 27 fetuses among the 8 pregnant females (Table 1). Eighteen fetuses from 5 females were estimated to be <60 d in gestational development, while 9 fetuses from 3 females were estimated to be >80 d (Table 2). The 9 full-term fetuses had fully developed internal organs, spotted hair, vibrissae, and nails, and ranged from 22.5–29.5 cm in crown-rump length (Figure 2d). The younger fetuses were <20 cm in length, lacked hair and had smooth, nearly transparent skin (Figure 2a and 2b). The 3 fetuses from "Female 3" had visible spotting on their skin although no hair was observed (Figure 2c).

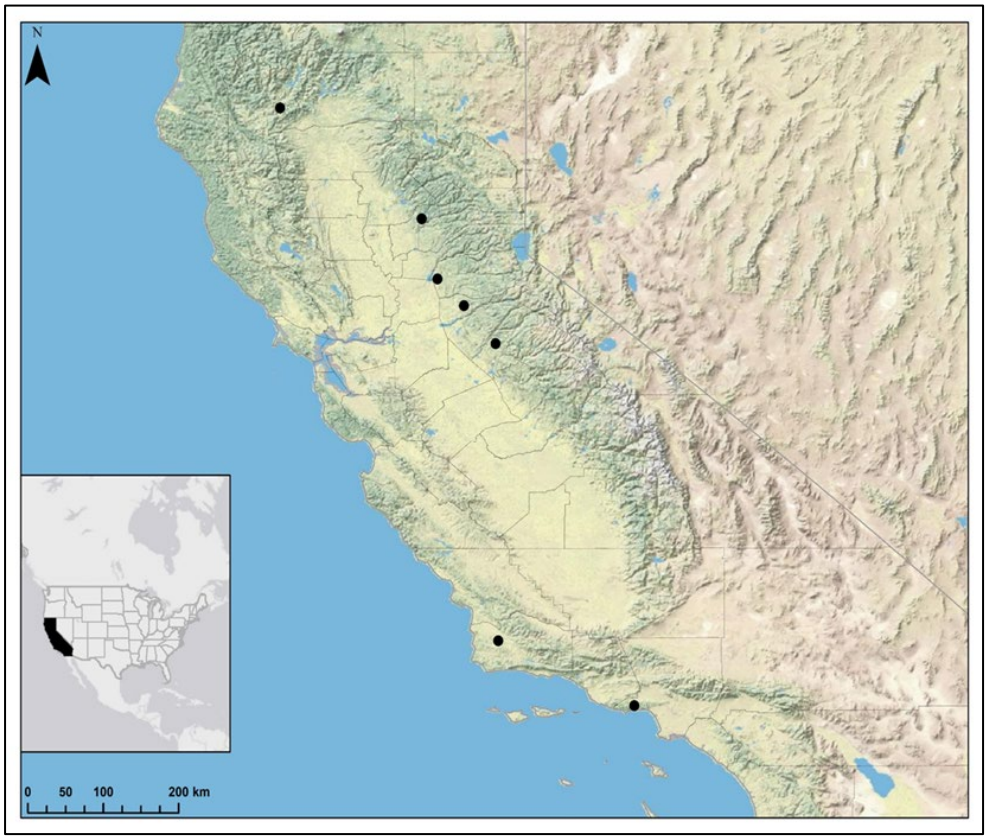

Figure 1. Mortality locations of the 8 pregnant female mountain lions (*Puma concolor*) submitted for necropsy in California, USA (2016–2023).

Table 1. Anticoagulant rodenticides (AR) detected in the livers of 8 pregnant female mountain lions (*Puma concolor*). Cause of death, location of mortality, age, body condition score (BCS), and number of fetuses each individual carried at the time of their death were recorded. Livers were tested for 7 ARs: brodifacoum (brd), bromadiolone (brm), chlorophacinone (chl), coumachlor (cou), difethialone (dif), diphacinone (diph), warfarin (war), and difenacoum (dfn). If an AR was not detected in the tested tissue, it was designated as "ND." For ARs, quantifiable concentrations are presented in parts per billions (ppb) and trace amounts indicate an AR was detected in the sample, but at a concentration below the reporting limit of 50 ppb. Concentrations for desmethylbromethalin (DMB) are not quantified, rather the presence or absence is detected (POS) or not detected (ND). Individuals that were not tested are designated with a dash (-).

Individual	Date of Death	Cause of Death	City	Cause of Death County	Age (years)	BCS	No. of Fetuses	Second Generation Anticoagulant Rodenticides				First Generation Anticoagulant Rodenticides				Neurotoxic Rodenticide
								BRD	BRM	DIF	DFN	CHL	COU	DIPH	WAR	DMB
Female 1	05/23/2016	Depredation	Sonora	Tuolumne	6	3	4	230	Trace	ND	ND	ND	ND	ND	ND	-
Female 2	02/21/2017	Vehicle strike	Los Alamos	Santa Barbara	4	3	2	71	130	Trace	ND	Trace	ND	830	ND	-
Female 3	03/08/2017	Depredation	Douglas Springs	Trinity	10	3	4	Trace	Trace	Trace	ND	ND	ND	Trace	ND	-
Female 4	04/29/2017	Depredation	Oregon House	Yuba	4	3	3	Trace	120	ND	ND	ND	ND	Trace	ND	-
Female 5	09/08/2019	Depredation	Sutter Creek	Amador	3	3	3	Trace	Trace	ND	ND	ND	ND	Trace	ND	-
Female 6	04/20/2023	Depredation	El Dorado Hills	El Dorado	5	3	4	Trace	Trace	ND	ND	Trace	ND	Trace	ND	ND
Female 7	09/20/2023	Depredation	Oregon House	Yuba	3	3	3	Trace	400	ND	ND	Trace	ND	400	ND	-
Female 8	06/17/2022	Vehicle Strike	Malibu	Los Angeles	5	3	4	83	930	190	ND	Trace	ND	230	ND	POS

Table 2. Anticoagulant rodenticides (AR) detected in the livers and placentas of mountain lion (*Puma concolor*) fetuses collected from 8 pregnant females necropsied between 2016 and 2023. Livers and placentas were pooled if less than 100 g of liver tissue could be collected (†). Livers and placenta were tested for 7 ARs: brodifacoum (brd), bromadiolone (brm), chlorophacinone (chl), coumachlor (cou), difethialone (dif), diphacinone (diph), warfarin (war), and difenacoum (dfn). If an AR was not detected, it was designated as "ND." For ARs, quantifiable concentrations are presented in parts per billions (ppb) and trace amounts indicate an AR was detected in the sample, but at a concentration below the reporting limit. Concentrations for desmethylbromethalin (DMB) are not quantified, rather presence or absence is detected (POS) or not detected (ND). Individuals that were not tested are designated with a dash (-). Stage of fetal maturation was estimated on size and appearance, inferring prenatal stages of development from domestic cats (*Felis catus*) (Knospe 2002).

Individual (fetus or placenta)	Mean Fetal Length (cm)	Estimated Fetal Maturation (days)	Second Generation Anticoagulant Rodenticides				First Generation Anticoagulant Rodenticides			
			BRD	BRM	DIF	DFN	CHL	COU	DIPH	WAR
Female 1(a,b,c,d)†	11	40-50	ND	ND	ND	ND	ND	ND	ND	ND
Female 2(a)	29.5	80-90	Trace	ND	ND	ND	ND	ND	150	ND
Female 2(b)	27.5	80-90	Trace	ND	ND	ND	ND	ND	160	ND
Female 3(a,b,c,d)†	18	50-60	ND	ND	ND	ND	ND	ND	ND	ND
Female 4(a,b,c)†	10.5	40-50	ND	ND	ND	ND	ND	ND	ND	ND
Female 5(a,b,c)†	11	40-50	ND	ND	ND	ND	ND	ND	ND	ND
Female 6(a,b,c,d)†	6.5	30-40	ND	ND	ND	ND	ND	ND	ND	ND
Female 6(e)†	NA	Placenta	ND	ND	ND	ND	ND	ND	Trace	ND
Female 7(a)	28.4	80-90	ND	ND	ND	ND	ND	ND	100	ND
Female 7(b)	28	80-90	ND	ND	ND	ND	ND	ND	86	ND
Female 7(c)	28	80-90	ND	ND	ND	ND	ND	ND	68	ND
Female 8(a)	25.5	80-90	Trace	Trace	ND	ND	Trace	ND	120	ND
Female 8(b)	25.5	80-90	Trace	Trace	ND	ND	Trace	ND	140	ND
Female 8(c)	22.5	80-90	Trace	Trace	ND	ND	Trace	ND	130	ND
Female 8(d)	24.5	80-90	ND	Trace	ND	ND	Trace	ND	120	ND

Figure 2. Necropsy photographs of mountain lion (*Puma concolor*) fetuses estimated to be (a) 30-40 d (from "Female 6"), (b) 40-50 d (from "Female 1"), (c) 50-60 d (from "Female 3"), and (d) 80-90 d (from "Female 7").

Despite all females having been exposed to ARs, only the 9 mature fetuses from "Female 2," "Female 7," and "Female 8" had detectable levels of ARs in their hepatic tissue (Table 2). Due to their size and development, the livers for each of the 9 fetuses were individually tested versus pooled. Both fetuses from "Female 2" had detectable levels of brodifacoum and diphacinone, while the 3 fetuses from "Female 7" only had detectable levels of diphacinone. Three of the 4 fetuses from "Female 8" were exposed to 4 different ARs; brodifacoum, bromadiolone, chlorophacinone, and diphacinone. The fourth fetus was exposed to 3 different ARs; bromadiolone, chlorophacinone, and diphacinone. Fetuses had varying concentrations of ARs in their tissues compared to their mother, and among each other, though in all cases where a compound was found in a fetus or the mother, the level was equal to (i.e., both trace; 2 cases) or higher, (i.e., trace vs. not detected, measurable vs. trace, or a larger residue; all other cases) in the mother (Tables 1 and 2). In the case of "Female 8," 3 of the 4 fetuses demonstrated exposure to brodifacoum in trace amounts, but the fourth fetus did not have detectable levels. The adipose of mountain lions "Female 6" and "Female 8" and the brain of their fetuses were tested for DMB. Only the adipose tissue of "Female 8" was positive for the toxic metabolite; none of her 4 fetuses had detectable levels of DMB, nor did "Female 6," her fetuses, or placentas.

Rodenticide toxicosis, due to lethal exposure to ARs or bromethalin, was not determined to be a contributing cause of mortality in any of the necropsied pregnant females nor was there evidence of coagulopathy or toxicosis in the fetuses. Exposure to ARs or bromethalin was not associated with a lower body condition – all adult females were observed to be in good body condition at the time of their death (BCS 3/5).

Discussion

Our findings demonstrate that AR exposure occurs during fetal development in mountain lions. Although we documented the presence of multiple ARs in the livers of the pregnant females and their mature fetuses, we did not observe evidence of coagulopathy. Felids have a higher tolerance to anticoagulants than other species (Petterino and Paolo 2001), which could explain why these individuals had such varying concentrations of exposure to multiple ARs and yet remained seemingly unaffected. Additionally, if maternal vitamin K plasma concentrations were normal throughout gestation, it is probable that adequate vitamin K crossed the placenta, enabling normal coagulation factors in the fetuses (Gupta and Gupta 2022). Moreover, we did not observe the presence of coumachlor, difenacoum, or warfarin in the tested samples. Unsurprising, coumachlor was not detected in any of the tested samples and has not been detected in free-ranging wildlife in California for a number of years (CDFW 2021, 2022, 2023) because it is not registered for use in the United States. Difenacoum and warfarin, however, are registered for use. While they were not detected in the current study samples, these 2 ARs have been previously detected in mountain lion livers in California (CDFW 2021, 2022, 2023, Rudd *et al.* 2018).

Although we did not observe toxicosis in this study, many toxicants can have an adverse sub-lethal impact on fetal growth or survival, often at doses that may not produce toxic effects on the mother (Gupta and Gupta 2022). For example, sub-lethal fetotoxic and teratogenic impacts such as birth deformities, low birth weight, slower growth rate, and delayed development have been reported in humans exposed to warfarin (Raivio *et al.* 1977; Hou 2004; Sathienkijkanchai and Wasant 2005; Mehndiratta *et al.* 2010; Starling *et al.* 2012; Yaqoob and Rubinstein 2019). Similar fetotoxic effects have been documented in pregnant animals exposed to ARs, as well as inappetence and lethargy (Mackintosh *et al.* 1988; Munday and Thompson 2003). These non-specific clinical signs are most likely associated with anemia and blood loss. If there were sub-lethal developmental impacts related to AR exposure in wildlife, they may not always be appreciated by solely examining remains. Voluntary neonate abandonment has been documented in female mountain lions, including populations in the SMMNRA (Moriarty *et al.* 2012; NPS unpublished data). Causes for abandonment can vary and may be related to maternal health, maternal experience, and the amount of maternal care that goes into rearing kittens (Engebretsen *et al.* 2024), pseudo-estrus (Benson *et al.* 2012), and neonate diseases such as panleukopenia. Given that the prevalence of AR exposure in necropsied mountain lions has been documented to be greater than 94% in California between 2016 and 2018 (Rudd *et al.* 2018; Rudd unpublished data), understanding if AR exposure produces sub-lethal fetotoxic effects that could influence maternal care and kitten survival merits additional attention.

Interestingly, only full-term fetuses had detectable levels of ARs in their livers, and at concentrations lower than those in their mothers. This could be attributed to differences in molecular structure and weight that could impede certain classes of ARs from freely moving through the placenta as quickly as others. Alternatively, Chetot *et al.* (2020a, b) examined the teratogenic effects of warfarin and bromadiolone in pregnant rats. Their findings demonstrated that warfarin induced teratogenic fetotoxic effects (Chetot *et al.* 2020a) and was transferred from the mother to the fetus during gestation or lactation, while bromadiolone was not (Chetot *et al.* 2020b). This was likely because of the nearly complete uptake of bromadiolone in the mother's liver,

preventing it from freely circulating into other tissues (Chetot *et al.* 2020b). Although 4 of the exposed fetuses from "Female 8" had trace levels of bromadiolone in their livers, only 3 of them had exposure to brodifacoum, highlighting the differences in maternal and fetal uptake.

The 8 pregnant mountain lions we examined were exposed to multiple ARs, including FGARs and SGARs. Although evidence of coagulopathy was not observed grossly or on histopathology, all mountain lions had evidence of extensive antemortem trauma, and cadavers were stored frozen until necropsy. Tissue damage due to trauma, decomposition, and freeze-thaw artifact could conceal minute lesions associated with coagulopathy. Further confounding a diagnosis of AR-related coagulopathy is the lack of threshold values associated with lethal and sub-lethal exposure (Lopez-Pera and Mateo 2018; Rattner and Harvey 2021).

One of the 2 mountain lions tested had exposure to bromethalin and detectable levels were not found in the fetuses. The inability to detect bromethalin in the exposed mountain lion fetuses could be due to an exposure event that occurred prior to pregnancy as retention time of bromethalin in select tissues is unknown. It is also possible that bromethalin does not cross the placenta. Adipose tissue has been prioritized to have the greatest diagnostic value when testing for bromethalin's metabolite, DMB, due to its lipophilicity. In animals that lack adipose, the high lipid content of the brain makes it a viable second choice when prioritizing samples for DMB testing. While other tissues such as liver and kidney may also be useful, results comparing exposure in various tissues of the same animal have varied (Bautista *et al.*, 2014; Romano *et al.* 2018; Murray and Cox 2023a). Diagnosing bromethalin toxicosis based on exposure to DMB alone is difficult because clinical signs can be non-specific, and severity of signs are often dose dependent. The bromethalin-exposed pregnant female was killed by vehicle strike – she had considerable trauma associated with the collision, so it is plausible that traumatic lesions could have obscured potential pathological brain lesions. However, pathological lesions are not always observed in cases of bromethalin toxicosis and freeze-thaw artifacts can obscure pathological assessments (Bautista *et al.*, 2014; McMillin 2016; Romano *et al.* 2018; Murray and Cox 2023a). We also cannot rule out that sub-lethal exposure may have caused impairment, putting this individual at risk of a collision.

Determining the source of rodenticide exposure for both ARs and bromethalin is difficult for many reasons. Mountain lions are opportunistic predators with a fair amount of plasticity in their diet (Moss *et al.* 2016) and have large home ranges that span nearly 400 km2 (Riley *et al.* 2021) across various geographical areas (Grigione *et al.* 2002). Exposure to anticoagulant rodenticides in non-target wildlife is more likely to occur in proximity to areas of human activity (Cypher *et al.* 2014; Serieys *et al.* 2015; Hindmarch and Elliot 2018; Lopez-Perea *et al.* 2019). However, Gabriel *et al.* (2012) showed that exposure and toxicosis to ARs, including other prohibited pesticides, also occurs on forested public and private lands due to illegal marijuana cultivation sites. Mountain lion home ranges include urban, suburban, agricultural, and forested habitats and they move around these areas within short periods of time. Additionally, ARs have long hepatic half-lives and bioaccumulate in tissues (Horak *et al.* 2018; Lopez-Pera and Mateo 2018), making it exceptionally challenging to identify time and place of exposure. While secondary exposure to ARs can occur, it is unclear if the same is true for bromethalin and DMB.

In conclusion, our findings provide evidence that ARs including brodifacoum, bromadiolone, chlorophacinone, and diphacinone can cross the placenta in a wild felid. Although there was no evidence of fatal toxicosis, deleterious sub-lethal impacts are largely unknown. Certain populations of mountain lions in California continue to remain isolated due to habitat fragmentation and largely impassible highways that serve as barriers to migration (Riley *et al.* 2014, 2021), including mountain lions in SMMNRA, where fatal AR toxicosis is the second most frequent form of human-caused mortality (Benson *et al.* 2020). These isolated populations are beginning to experience low genetic diversity (Riley *et al.* 2014) and declines in reproductive health due to inbreeding (Ernest *et al.* 2014; Huffmeyer et al 2022), so ensuring the survival and recruitment of healthy individuals is imperative for the species survival in these isolated areas. Further monitoring and documentation of declines in reproductive fitness that may be associated with AR exposure are warranted, as are further studies evaluating the impacts of sub-lethal bromethalin exposure. Surveillance programs could also monitor trends associated with current and new legislation, including the recent passage of California Assembly Bill 1322 which was implemented in January 2024, that prohibits the use of diphacinone in a similar manner as the SGARs. Future research should also focus on monitoring targeted rodent pest populations which may provide better resolution for efficacy studies as to when and where rodenticides (both ARs and non-ARs) are being used in relation to current regulations and legislation. Additionally, evaluating tissues for cis and trans isomers of ARs, especially for SGARs which are more persistent in liver would be beneficial. Trans isomers for difenacoum (Damin-Pernik *et al.* 2016), difethialone (Lefebvre *et al.* 2020), and brodifacoum (Fourel *et al.* 2021) have significantly shorter half-lives in animal tissues, potentially reducing the risk of

secondary exposure and toxicosis in non-target wildlife, especially in use sites exempt from AR restrictions.

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Dr. Robert Poppenga is professor of Clinical Veterinary Toxicology and Section Head, Toxicology Laboratory at the California Animal Health and Food Safety Laboratory (CAHFS), School of Veterinary Medicine, University of California at Davis. He has been at UCD since 2004. He received his DVM and PhD degrees from the University of Illinois. He was a staff veterinarian at the first national animal poison control center from 1983 through 1987. He is board-certified by the American Board of Veterinary Toxicology and has served that organization in a number of roles including President. He has almost 35 yrs of experience as a clinical and diagnostic veterinary toxicologist including previous faculty and diagnostic laboratory positions at Michigan State University and the University of Pennsylvania. He is author or co-author on over 160 scientific manuscripts and textbook chapters. He is active member in numerous professional organizations including the American Association of Veterinary Laboratory Diagnosticians (former Executive Board

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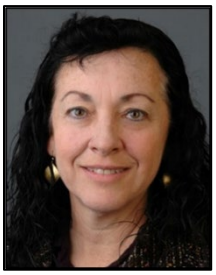

Dr. Seth Riley grew up in Washington, DC where he first got interested in wildlife, by way of snakes. He graduated in 1988 from Stanford University with a BA. in Human Biology, concentrating in Animal Behavior and Ecology. From 1988–1990, Seth worked as a wildlife biologist for the National Park Service at the Center for Urban Ecology in Washington. He worked on a number of urban wildlife issues there, focusing particularly on the behavioural, population, and disease ecology of urban raccoons. Seth then went to the University of California, Davis for graduate school, where he graduated with a PhD in Ecology in 1999. His dissertation work was on the ecology of bobcats and gray foxes in urban and rural areas of Golden Gate National Recreation Area, just north of San Francisco. After graduating Seth worked as a post-doctoral fellow at Davis studying hybridization between native and introduced tiger salamanders in the Salinas Valley of California. At the beginning of 2000, Seth began in his current position as Wildlife Ecologist with the National Park Service at Santa Monica Mountains National Recreation Area in southern California. His current projects, all related to the impacts of urbanization and fragmentation on wildlife, include long-term bobcat and mountain lion telemetry studies, stream surveys for amphibians, pitfall/drift fence trapping to determine terrestrial reptile and amphibian distribution and abundance, and a number of projects on the impacts of freeways on wildlife. Seth also has an adjunct position at UCLA where he advises students and teaches graduate seminars. Seth co-edited a book on Urban Carnivores, for which he co-authored 7 chapters, which came out in 2010, and a book on the effects of roads on smaller wildlife species, which came out in 2015.

Jeff Sikich is a biologist with the National Park Service researching the impacts of urbanization and habitat fragmentation on mountain lions in Southern California. Jeff manages the field work for this study, one of the longest running of its kind, which informs mountain lion conservation, as well as outreach, education, and habitat conservation efforts locally and around the world. In addition to his work in California, Jeff has contributed to mountain lion research in Montana, Arizona, Mexico, Nicaragua, and Peru. He is a graduate of Indiana University with a degree in Environmental Science and Management. Over the last 26 yrs, Jeff has captured and handled over 16 carnivore species for wildlife research. His work specializes in safe capture and immobilization techniques for large carnivores, and he has advised and trained many professionals around the world on humane capture methods. He has worked on several projects in the United States researching a variety of species, including gray wolves, black bears, lynx, coyotes, bobcats, desert tortoise, and deer, as well as studies focused on tigers in Sumatra, jaguars in North, Central, and South America, and leopards in South Africa. His worked has centered on conservation, including habitat connectivity, corridors, toxicants and human-wildlife conflict resolution. He has contributed to many scientific papers and popular articles on carnivore ecology and conservation, and his work has been featured on numerous television programs and news outlets including National Geographic, PBS Nature, 60 Minutes, The New Yorker, The Atlantic, The Wall Street Journal, Washington Post, The Guardian, New York Times, Los Angeles Times, NPR, AP News, and more.

Dr. Nicolás Streitenberger obtained his DVM (2012) and PhD (2020) degrees from the National University of La Plata, Argentina. His PhD was on Bovine Respiratory Disease in feedlot cattle, with particular interest on viral infections. From 2020 to

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