Department of Pesticide Regulation
Pesticide Registration Branch
November 16, 2018

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

Introduction

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

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

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from a variety of sources, including peer-reviewed scientific publications, statewide sales and use reporting data, and unpublished wildlife incident and mortality data.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

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

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

Background

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

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

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

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

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

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

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

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

[a] Data summarized from DPR, 2013

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

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

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

[a] Data summarized from DPR, 2013

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

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

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

Descriptions of Data and Exhibits Submitted to DPR by Michael Graf

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

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

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

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

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

Notes:

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

ARs: Anticoagulant Rodenticides

FGARs: First Generation Anticoagulant Rodenticides

SGARs: Second Generation Anticoagulant Rodenticides

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

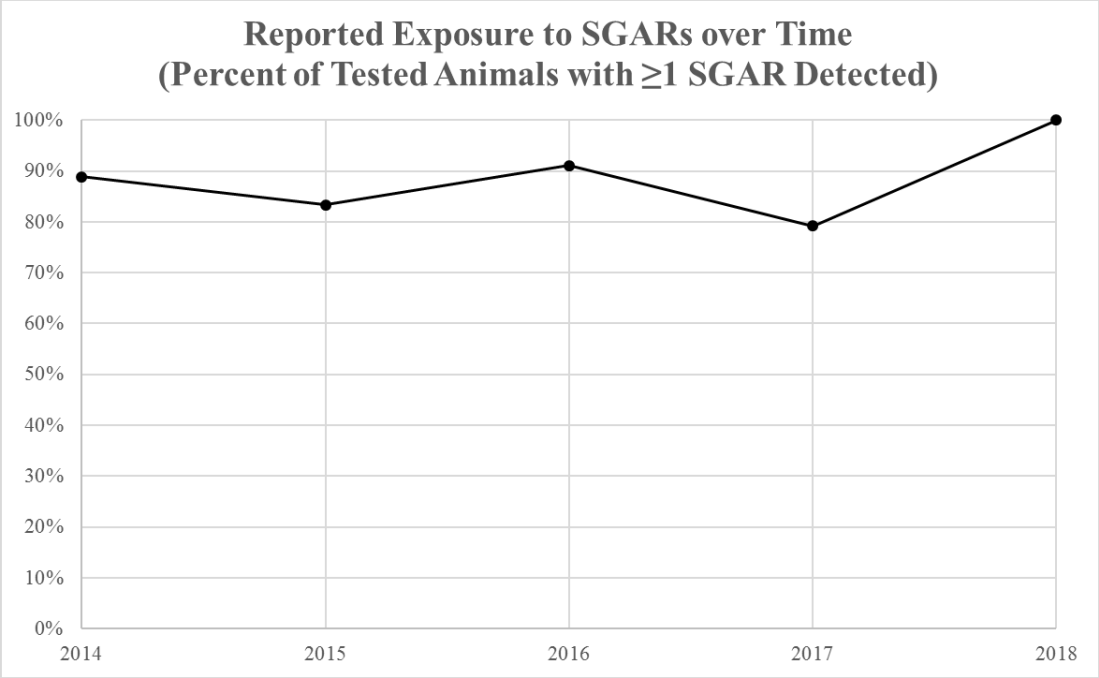


Figure 2 – Exposure rates of individual SGAR active ingredients from 2014-2018 (chart created by DPR scientists from non-target wildlife loss reports submitted by DFW).

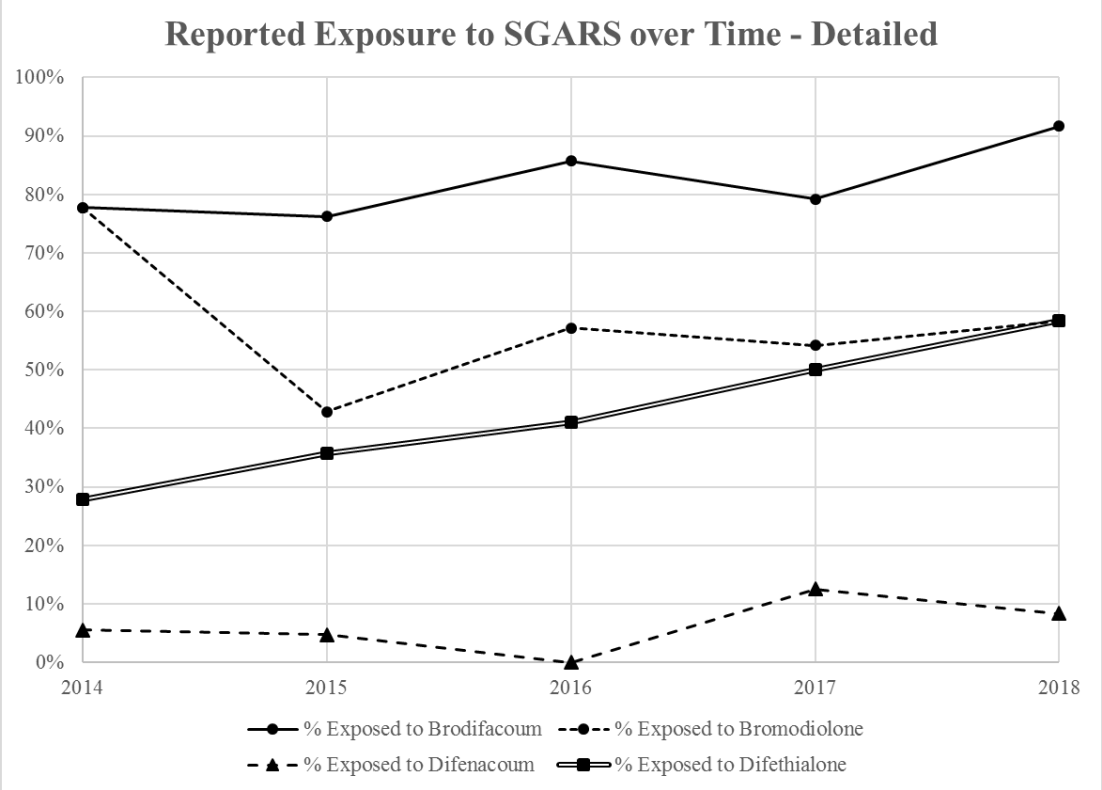


Figure 3 – DPR's preliminary analysis of FGAR non-target wildlife exposure rates based on loss reports submitted by DFW.

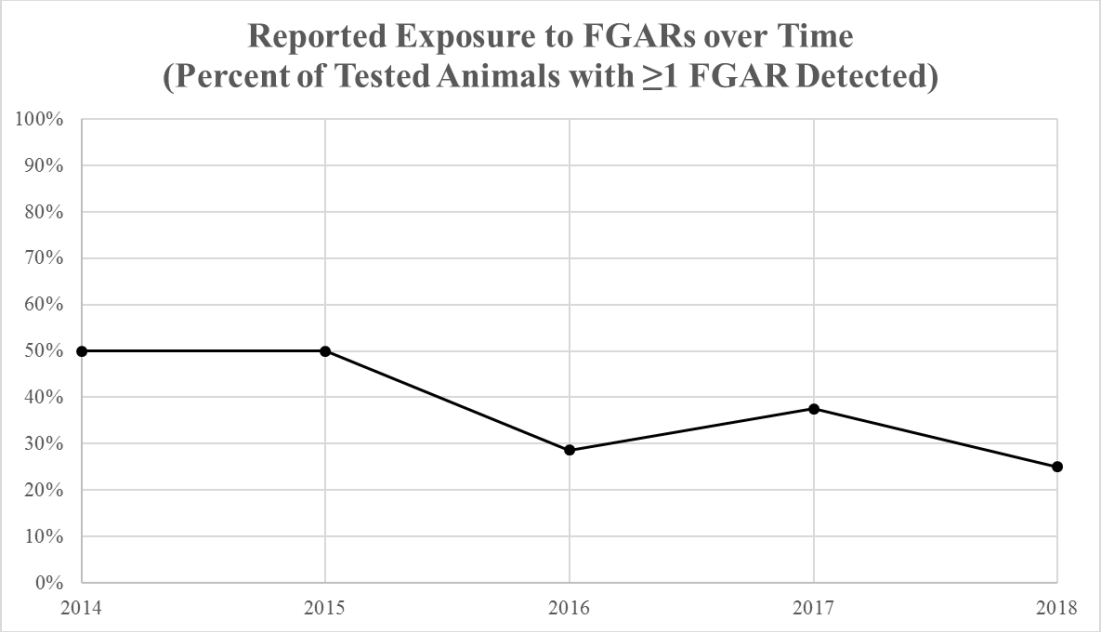


Figure 4 – Exposure rates of individual FGAR active ingredients from 2014-2018 (chart created by DPR scientists from non-target wildlife loss reports submitted by DFW).

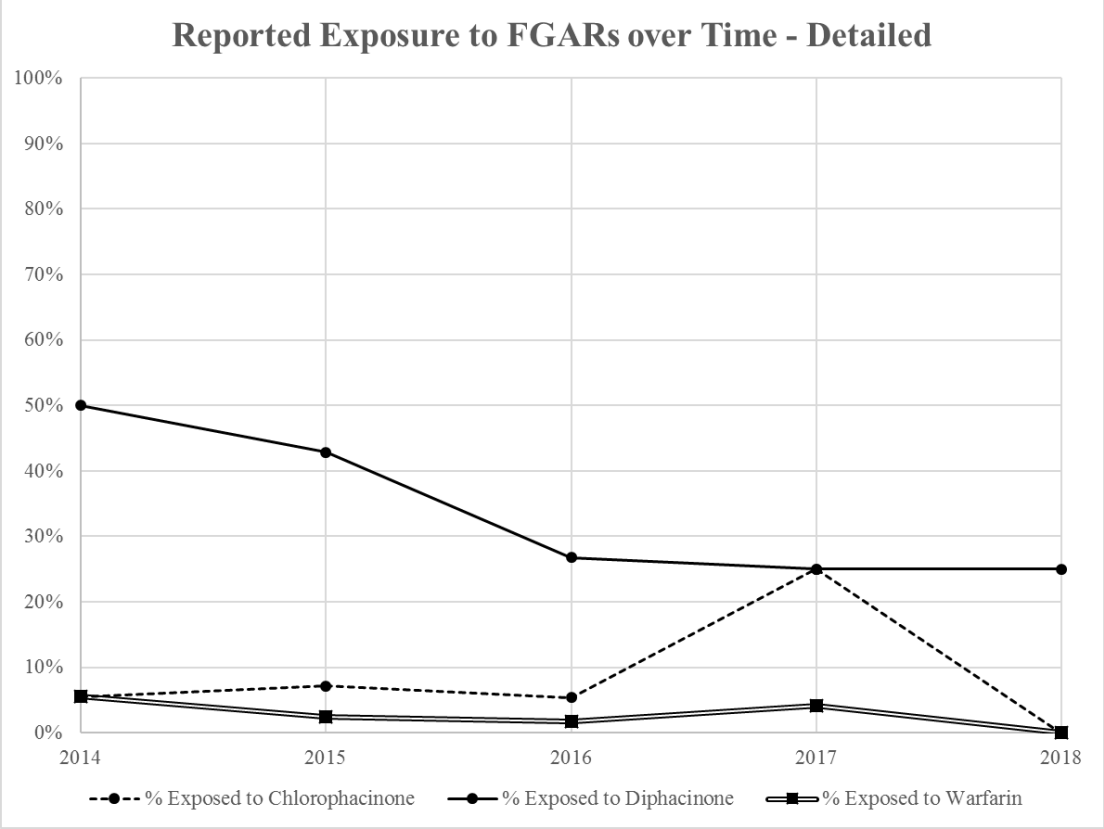
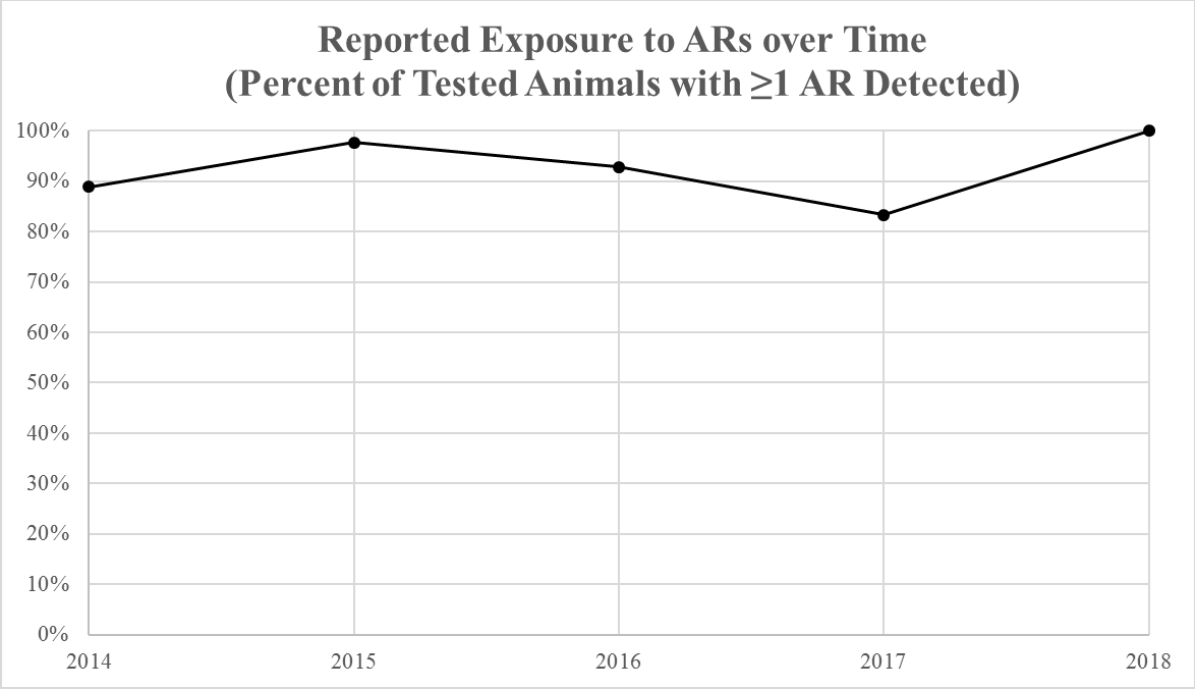


Figure 5 – DPR's preliminary analysis of AR (all ARs, 1st and 2nd generation) exposure rates based on non-target wildlife loss reports submitted by DFW.



- **DFW Mountain Lion Database**

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

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

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

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

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

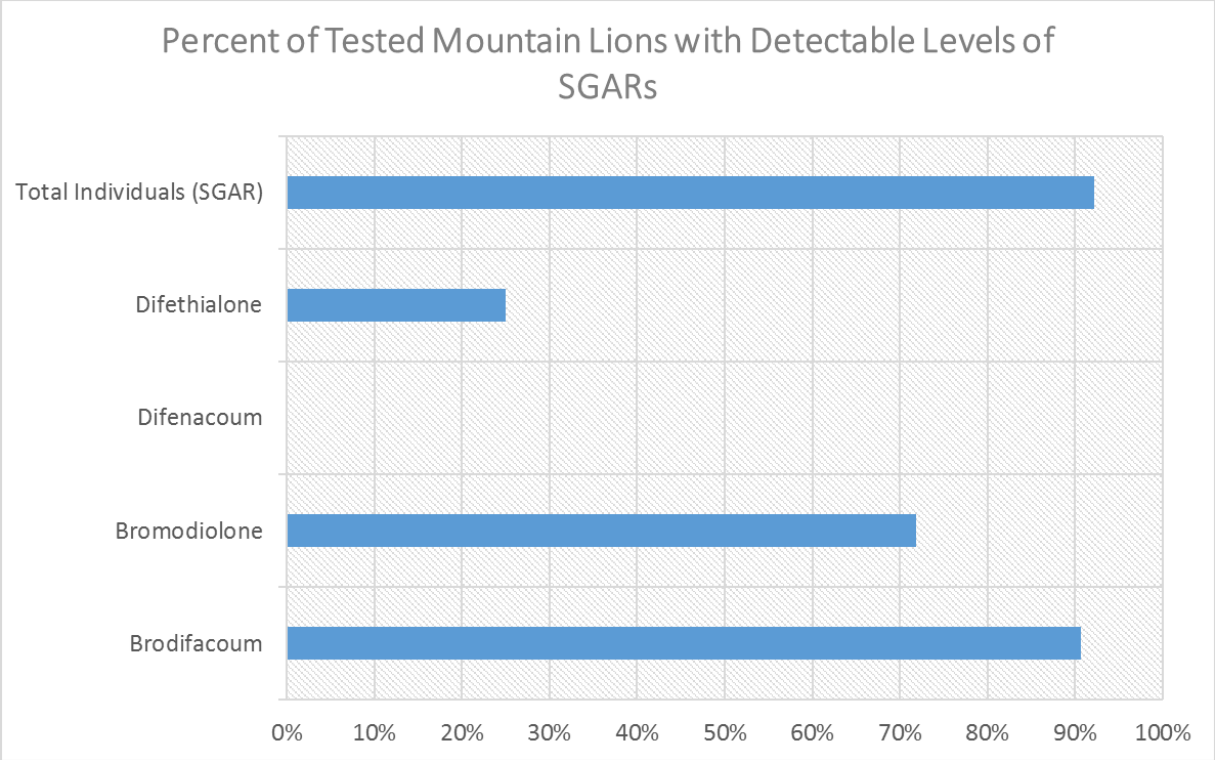
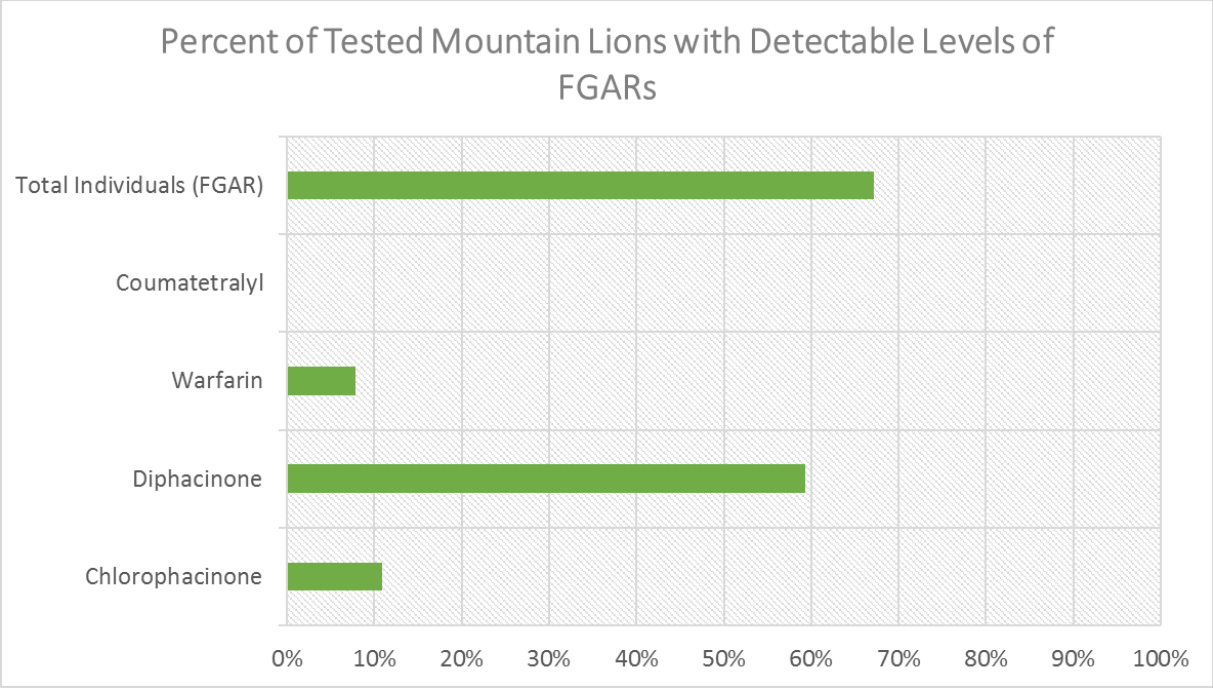


Figure 7 – First-generation anticoagulant rodenticide (FGAR) exposure rates among tested mountain lions (bar graph created by DPR scientists using DFW data).



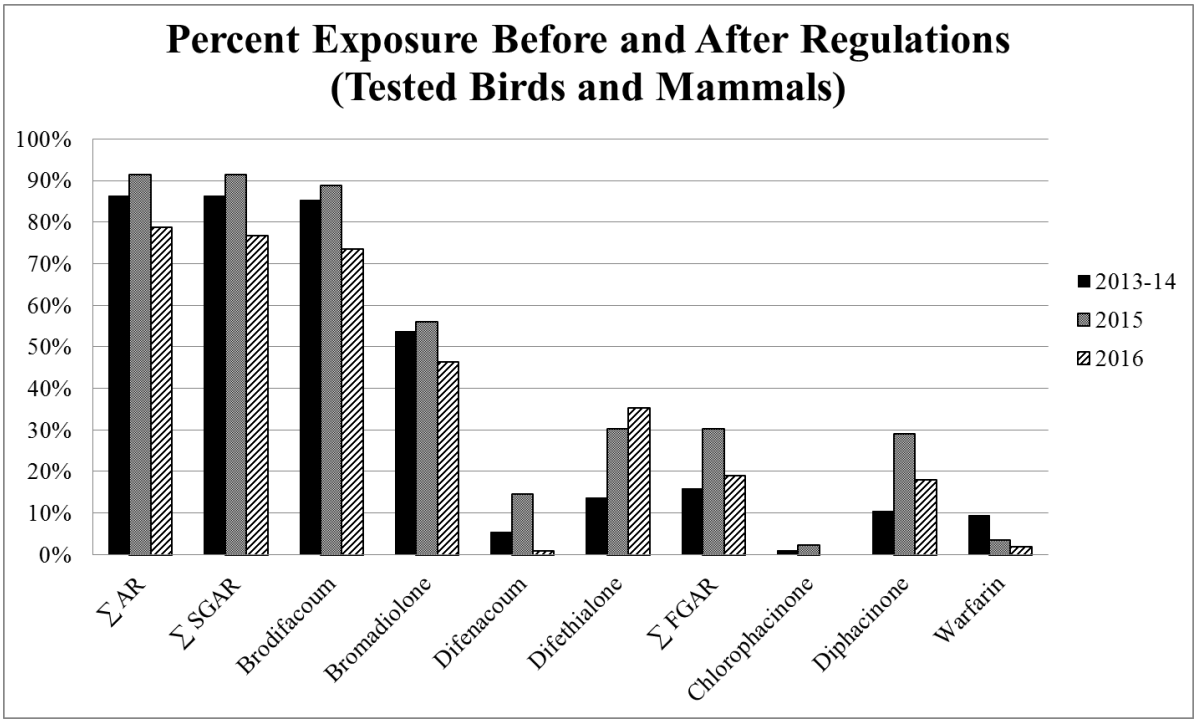
- **WildCare Wildlife Rehabilitation Center Data**

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In another portion of the manuscript the study authors stated that

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

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

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

The study authors concluded that:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 4

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

Summaries of AR Data and Information from Regulatory Agencies

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

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

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

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

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

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

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

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

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

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

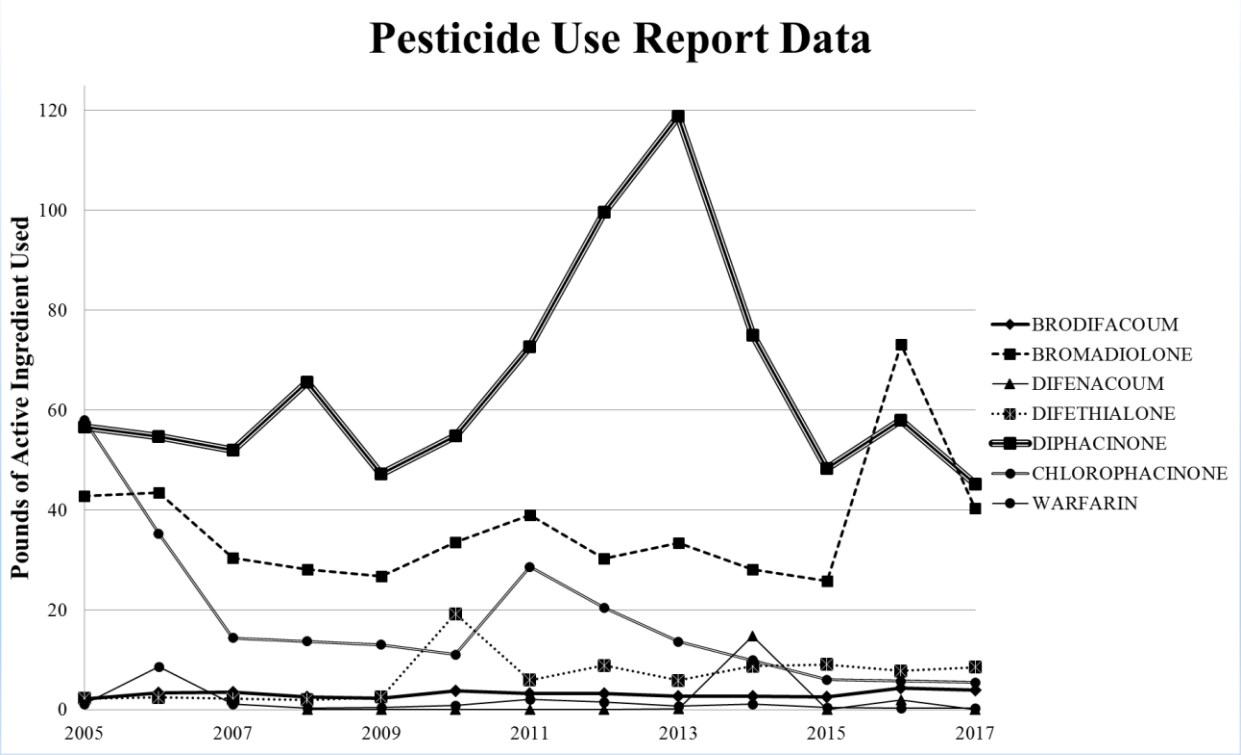
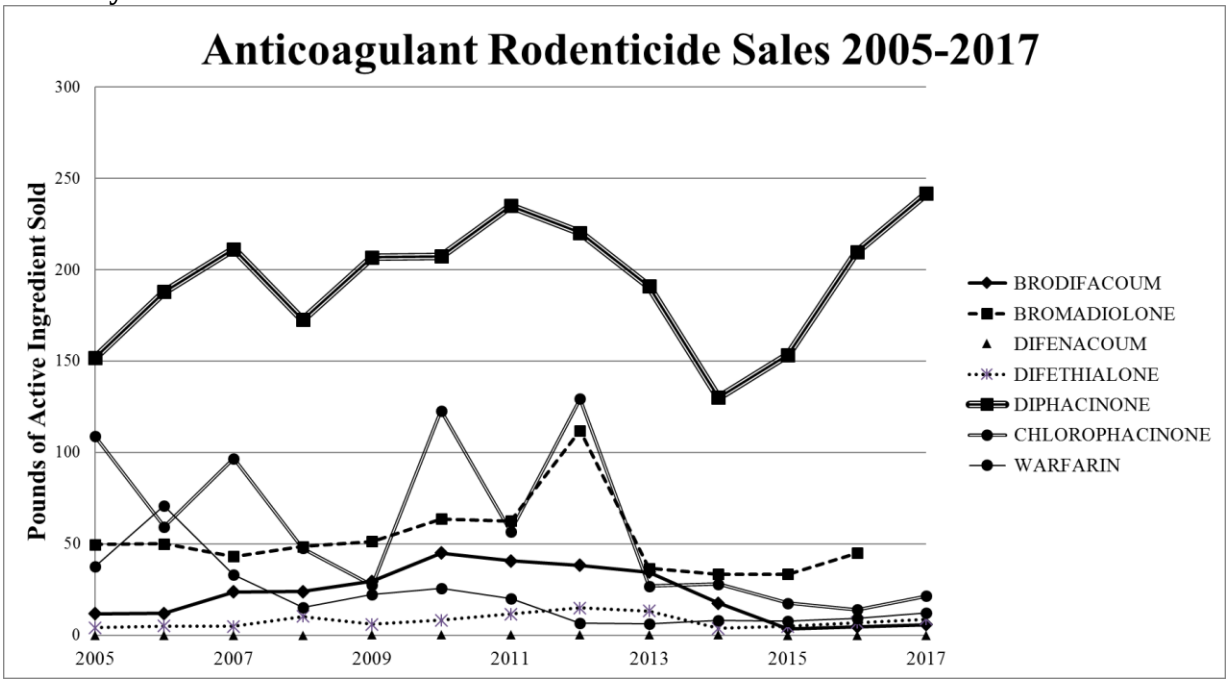


Figure 10 – A summary of AR sales data from 2005-2017. Sales data for bromadiolone in 2017 indicated that 638 pounds of active ingredient was sold. This is most likely an error, so 2017 sales data for bromadiolone is not present in this graph. DPR sales reports are based on information obtained from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



Conclusion

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

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

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

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

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

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

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

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

References

CAHFS (California Animal Health and Food Safety Laboratory), 2015, Necropsy report for a skunk. CAHFS Case #: D1503125 (Internal report submitted to DPR through the WildCare contract [2013-2016]).

CDFW, 2015, Report to the Fish and Game Commission: A Status Review of the Fisher (*Pekania* [Formerly *Martes*] *Pennanti*) in California, May 12, 2015, Available via <https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=101470>, accessed October 18, 2018.

DPR, 2013, Second Generation Anticoagulant Rodenticide Assessment, June 27, 2013, Available via < https://www.cdpr.ca.gov/docs/registration/reevaluation/chemicals/brodifacoum_final_assess.pdf >, accessed October 17, 2018.

DPR, 2014, Notice of Final Decision Concerning Brodifacoum (Second Generation Anticoagulant Rodenticide), July 18, 2014, Available via <https://www.cdpr.ca.gov/docs/registration/canot/2014/ca2014-09.pdf >, accessed October 17, 2018.

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

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

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

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

Majerus, P. W., and D. M. Tollefsen, 2006, Blood Coagulation and Anticoagulant, Thrombolytic, and Antiplatelet Drugs, In: L. L. Brunton, Ed., The Pharmacological Basis of Therapeutics, 11th Edition, The McGraw-Hill Companies, New York.

Novak, K., Torfeh, D., 2017, Raptor Pilot Study for Levee Protection - Integrated Pest Management Program, Ventura County Public Works Agency, Watershed Protection District,

available via: <https://vcportal.ventura.org/BOS/District2/RaptorPilotStudy.pdf>, accessed October 16, 2018.

Ott, R.L., Longnecker M., 2010, An Introduction to Statistical Methods and Data Analysis, 6th Edition, Brooks/Cole Publishers: Belmont, CA, pp. 1273.

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

Redfern, R., Gill, J.E., & Hadler, M.R, 1976, Laboratory Evaluation of WBA 8119 as a Rodenticide for Use Against Warfarin-Resistant and Non-Resistant Rats and mice, J Hyg Camb, 77: 419-426.

Saravanan, K., and Kanakasabai, R., 2004, Evaluation of Secondary Poisoning of Difethialone, a new second-generation anticoagulant rodenticide to Barn Owl, *Tyto alba* Hartert under captivity, Indian Journal of Experimental Biology, 42: 1013-1016.

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

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

Thomson, W.T., 1988, Agricultural Chemicals, Book III - Miscellaneous chemicals: Fumigants, growth regulators, repellents, and rodenticides (1988/89 Revision), Fresno, California, Thomson Publications.

U.S. EPA, 2004, Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: a Comparative Approach, EPA-HQ-OPP-2004-0033-0004.

U.S. EPA, 2007, EFED Risk Assessment for the Proposed Section 3 Registration of Difenacoum for Control of Norway Rats, Roof Rats, and House Mice, EPA-HQ-OPP-2009-0081-0137.

U.S. EPA, 2012, Ecological Risk Assessment for the Section 3 New Use Registration of Diphacinone (PC 067701) For Use on Black-tailed Prairie Dogs (*Cynomys ludovicianus*), EPA-HQ-OPP-2012-0739-0002.

U.S. EPA, 2015-a, Preliminary Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species, and Drinking Water Assessments in Support of the Registration Review of Chlorophacinone, EPA-HQ-OPP-2015-0778-0004.

U.S. EPA, 2015-b, Preliminary Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species, and Drinking Water Assessments in Support of the Registration Review of Warfarin, EPA-HQ-OPP-2015-0481-0009.

U.S. EPA, 2016-a, Brodifacoum: Human Health Risk Scoping Document in Support of Registration Review, EPA-HQ-OPP-2015-0767-0003.

U.S. EPA, 2016-b, Bromadiolone: Human Health Seeping Document in Support of Registration Review, EPA-HQ-OPP-2015-0768-0003.

U.S. EPA, 2016-c, Problem Formulation for the Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments in Support of the Registration Review for Difethialone, EPA-HQ-OPP-2015-0770-0002.

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