2018

Anticoagulant Rodenticide Residues in Game Animals in California

Stella C. McMillin

Robert H. Poppenga

Shannon C. Chandler

Deana L. Clifford

Anticoagulant Rodenticide Residues in Game Animals in California

Stella C. McMillin
Wildlife Investigations Laboratory, California Department of Fish and Wildlife, Rancho Cordova, California
Robert H. Poppenga
California Animal Health and Food Safety Laboratory, Davis, California
Shannon C. Chandler
USDA/APHIS/Wildlife Services, Sacramento, California
Deana L. Clifford
California Department of Fish and Wildlife, Gold River, California

ABSTRACT: Anticoagulant rodenticides (ARs) are used to control rodents around homes, buildings, and in agriculture. They have been found widely in predatory and scavenging wildlife as a result of secondary exposure and less commonly in herbivores and omnivores from primary exposure. While predators and scavengers have been monitored for AR exposure, very little information is available about AR residues in edible muscle tissue of game animals. Game animals may be exposed to ARs through direct consumption of bait, ingestion of contaminated food or vegetation, or consumption of contaminated prey items. Carcasses of three species of game animals (black bear, wild pigs, and mule deer) were collected opportunistically for this study from 2013 to 2015. Causes of death were mainly depredation, vehicular trauma, or hunter harvest. Sampling was performed in the field by California Department of Fish and Wildlife and US Department of Agriculture Wildlife Services staff. Tissues were analyzed for 37 deer in 11 counties, 120 wild pigs in 10 counties, and 12 bears in eight counties. The highest prevalence of AR exposure was found in bears, with 83% of tested livers containing ARs. Bear were most likely to be exposed to brodifacoum, a second generation AR used primarily in and around residences. Prevalence of exposure in wild pigs was 8.3%. Pigs were most likely to be exposed to chlorophacinone, used primarily in agriculture. More than half of pigs with AR residues in their liver also had AR residues in their muscle tissue. There were no AR detections in muscle samples tested for bears with AR residues in liver. None of the deer livers tested positive for ARs.

KEY WORDS: anticoagulant rodenticide, bear, deer, game animals, non-target exposure, wild pig, swine

Proc. 28th Vertebr. Pest Conf. (D. M. Woods, Ed.)
Published at Univ. of Calif., Davis. 2018. Pp. 243-246.

INTRODUCTION

Exposure of wildlife to anticoagulant rodenticides (ARs) has been widely documented in California in a diversity of taxa and in a variety of habitats (Hosea 2000, Riley et al. 20005, Gabriel et al. 2012, Cypher et al. 2014, Serieys et al. 2015). Most monitoring studies have focused on determining exposure in predators and scavengers, and there has been no routine monitoring of game species in California. Game animals may be tested for ARs, particularly when hunters discover discoloration in the muscle or fat of their harvested animals (e.g., "blue pigs") presumably due to dyes added to anticoagulants. In this case, muscle or fat tissue is tested to determine if consumption of the meat poses a hazard to human health. However, as demonstrated by findings of exposure in non-game animals, the discoloration is not always present and is not a reliable indicator of AR residues in meat.

ARs cause mortality by binding to enzymes responsible for recycling vitamin K and thus impairing an animal's ability to produce several key clotting factors. There is a lag time of several days between ingestion and death during which they may be at an increased risk for predation. ARs fall into two categories based on toxicological characteristics and use patterns. Older, first-generation ARs (FGARs) were developed and made available in the 1950s. Products with these ingredients are less toxic than second-generation anticoagulants (SGARs) and require consecutive days of intake to achieve a lethal dose. They also have a lower ability to accumulate in biological tissue and clear more rapidly, with liver half-lives (depending on the particular compound) of 2 to 26 days (Erickson and Urban 2004, Fisher et al. 2003). FGARs are used both for commensal and field rodent control. SGARs have liver half-lives of >80 to 350 days (Erickson and Urban 2004) and are typically used only for control of commensal rodents.

Game animals may be exposed to ARs through direct consumption of bait, ingestion of contaminated food or vegetation, or consumption of contaminated prey items. Deer would most likely to be exposed directly through consumption of bait as was observed in New York (Stone et al. 1999) or through intentional poisoning (Gabriel et al 2013). Wild pigs and bear, being opportunistic omnivores, could be exposed through additional routes, including consumption of contaminated rodents or small invertebrates (Boothe et al. 2003, Elliott et al. 2013) and consumption of refuse. ARs have been documented to occur around marijuana cultivation sites on public lands, placed particularly around young plants to discourage herbivory and damage to irrigation lines (Gabriel et al. 2012). This may be a major source of exposure as 600 large-scale marijuana cultivation sites were reported to occur on only two of California's 17 national forests (Gabriel et al. 2013).

In order to assess exposure potential to hunters and others who consume hunted meat, it is necessary to determine concentrations of ARs in edible tissues of game animals. AR residues concentrate in the liver but may also be found in other body tissues. In feral pigs dosed with SGAR brodifacoum, livers contained the highest concentrations but muscle tissue also contained brodifacoum (Eason et al. 2001). In rats, chlorophacinone,

Table 1. Compounds analyzed in anticoagulant rodenticide scan with reporting limits.

SGAR/FGAR	Compound	Reporting Limit (ppm), Muscle and Liver	Reporting Limit (ppm), Blood
FGAR	Chlorophacinone	0.020	0.001
	Coumachlor[1]	0.020	0.001
	Diphacinone	0.020	0.001
	Warfarin	0.020	0.001
SGAR	Brodifacoum	0.020	0.001
	Bromadiolone	0.020	0.001
	Difenacoum	0.020	0.001
	Difethialone	0.020	0.001

a FGAR, was detectable in all tested body tissues (liver, kidney, lung, heart, muscle, and fat) 48 hours after exposure (Eason et al. 2001). In addition to potential for human exposure, AR exposure in game animals has the potential to spread through the food chain and expose other wildlife. Monitoring of mammalian predators and scavengers, such as coyotes, mountain lions, and bobcats (Riley 2007) confirm widespread exposure, as does monitoring of avian predators and scavengers (Lima and Salmon 2010, Kelly et al. 2014).

METHODS

Carcasses of threes species of game animals [black bear (*Ursus americanus*), wild pigs (*Sus scrofa*), and mule deer (*Odocoileus hemionus*)] were collected opportunistically for this study. Causes of death were mainly depredation, vehicular trauma, or hunter harvest. Sampling kits were provided to CDFW scientific and enforcement staff in northern and central California, and USDA Wildlife Services staff. Tissue samples (liver, muscle, and blood) were extracted in the field and sent with accompanying collection data to the Wildlife Investigations Laboratory.

Samples were kept frozen until they could be analyzed. Four SGAR (brodifacoum, bromadiolone, difethialone, and difenacoum) and four FGAR (chlorophacinone, diphacinone, warfarin, and coumatetralyl) compounds were analyzed (Table 1). Samples were screened for ARs using liquid chromatography-tandem mass spectrometry and positive samples were quantitated with high-performance liquid chromatography. When ARs were detected in a liver sample, the muscle sample and/or blood sample from the same animal was submitted for analysis. For a few samples, the reporting limits were slightly higher because less tissue was available for analysis.

RESULTS

Sample kits were received for a total of 17 black bears, 38 mule deer, and 123 pigs. Not all sampling kits were returned with all requested tissues. All sampling kits containing liver samples were analyzed.

Black Bear

Sampling kits for 17 black bears from eight counties were returned (Table 2). Of these, 12 kits contained liver samples. Eight of these bears were killed for depredation, two were hit by cars and one appeared to die of illness. Ten

Table 2. Black bear anticoagulant rodenticide results.

Date	County	COD	Liver AR (ppm)	Muscle AR (ppm)	Blood AR (ppm)
7/12/13	El Dorado	Illness	BROD 0.046 BROM 0.67	ND	NT
8/8/13	Madera	Hit by car	BROD trace*	ND	NT
9/13/13	Tehama	Hit by car	ND	NT	NT
12/3/13	Mendocino	Hit by car	ND	NT	NT
7/8/14	Kern	Depredation	CHLOR trace	ND	ND
7/9/14	Tuolumne	Depredation	BROD trace	ND	NT
7/22/14	Tuolumne	Depredation	CHLOR trace	ND	NT
7/30/14	SLO	Depredation	BROD trace, CHLOR trace	ND	NT
10/7/14	Kern	Depredation	BROD trace, DIPH 1.1	ND	trace
10/8/14	Kern	Depredation	BROD 0.61	NT	ND
1/5/15	Placer	Depredation	BROD 1.5 BROM 1.5 DIFETH 5.7 DIFEN trace	NT	ND
9/17/15	Madera	Hit by car	BROD trace	ND	ND

Table 3. Wild pig anticoagulant rodenticide detections by county.

County	Number of Samples	Percent Positive
Humboldt	1	0
Kern	2	0
Mariposa	42	5
Mendocino	2	0
Nevada	4	0
Placer	2	0
San Luis Obispo	61	13
Sonoma	2	0
Sutter	2	0
Tuolumne	2	0

Table 4. Wild pig anticoagulant rodenticide results.

Date Collected	County	Liver AR	Muscle AR
7/9/14	SLO	CHLOR 0.32	ND
7/11/14	SLO	CHLOR 0.077	CHLOR trace
7/11/14	SLO	CHLOR trace*	CHLOR trace
7/11/14	SLO	CHLOR trace	ND
7/11/14	SLO	CHLOR 0.15	CHLOR trace
7/17/14	SLO	BROD trace	ND
7/30/14	SLO	BROD trace DIPH 0.069	BROD trace
8/21/14	SLO	CHLOR trace	ND
9/17/14	Mariposa	BROD trace	NT
4/1/15	Mariposa	BROD trace	ND

*Trace = detected below the reporting limit,
SLO = San Luis Obispo, CHLOR = chlorophacinone, BROD = brodifacoum, DIPH = diphacinone,
ND = not detected, NT = not tested

of twelve liver samples were positive for anticoagulant rodenticides, with brodifacoum being detected most frequently. For positive liver samples, eight kits containing muscle samples were tested. All were negative. Blood samples were tested for five of the kits with positive liver samples. Trace brodifacoum was in one blood sample.

Wild Pigs

Sampling kits for 123 wild pigs were returned with 120 containing liver samples. Samples were submitted from 10 counties; 84% came from Mariposa and San Luis Obispo Counties (Table 3). Cause of death for all pigs was depredation. Ten liver samples were positive for Ars; most commonly chlorophacinone (Table 4). For the ten positive liver samples, corresponding muscle samples were available for nine. Four of the nine muscle samples also tested positive for ARs. Chlorophacinone was the most likely to be found in muscle tissue, with 50% of muscle tissue samples with corresponding chlorophacinone-containing liver samples also testing positive.

Mule Deer

Sampling kits for 37 mule deer from 11 counties were returned, all containing liver samples. Causes of death for the deer varied: 22 were physical trauma (typically vehicular), six were unknown, five were hunter harvested, and five died from illness. None of the liver samples tested positive for ARs (Table 5). Corresponding blood samples were tested for 16 deer and all were negative for ARs.

DISCUSSION

Given the dietary preferences of mule deer, it is not surprising that no ARs were detected in the liver tissue of the 37 liver samples and 16 blood samples that were tested. The existence of numerous trespass cannabis grows in the area where the majority of the samples were taken did, however, present increased risk for rodenticide exposure. As deer are the most commonly hunted and consumed big game animal in California, with 21,749 deer harvested in California in 2017 (CDFW 2017 Deer Harvest Statistics), contaminated deer tissue would be of utmost concern.

A relatively small number of bear tissues were analyzed for this study, and results were similar to opportunistic sampling previously done by CDFW (CDFW files). It is important to note that the majority of

Table 5. Summary of anticoagulant rodenticide detections from bear, pig, and deer.

	Bear			Pig		Deer	
	Liver	Muscle	Blood	Liver	Muscle	Liver	Blood
Brodifacoum	8/12	0/7	1/4	4/120	1/9	0/37	0/16
Bromadiolone	2/12	0/7	0/4	0/120	0/9	0/37	0/16
Difethialone	1/12	0/7	0/4	0/120	0/9	0/37	0/16
Difenacoum	1/12	0/7	0/4	0/120	0/9	0/37	0/16
Chlorophacinone	3/12	0/7	0/4	6/120	3/9	0/37	0/16
Diphacinone	1/12	0/7	0/4	1/120	0/9	0/37	0/16
Warfarin	0/12	0/7	0/4	0/120	0/9	0/37	0/16

bears sampled in this study were taken for depredation, indicating interaction with people, and increased risk of exposure to ARs. While bears are not as commonly hunted or consumed as deer, 1,417 bears were harvested in the 17/18 bear season (CDFW Bear Season Statistics). The highest concentration of total ARs in the bear livers tested was 8.7 ppm from a bear taken for depredation in Placer County. Even at this high concentration, a 60 kg person would need to consume almost 2.68 kg of liver (about 6 lbs) to consume the mammalian LD_{50} (0.39 mg/kg body weight for rats) of brodifacoum (the most toxic of the ARs). However, monitoring of AR exposure in bears harvested by hunters is advised to determine the potential for exposure in hunters consuming bear tissues.

The finding of ARs in livers of wild pigs is not surprising. Wild pigs have a varied diet, which includes both animal and plant matter and cause a considerable amount of damage to farms and ranches, and may be exposed to pesticides used in these areas. The greatest numbers of wild pigs in California are found west of the Central Valley from Mendocino to San Luis Obispo. FGARs were more likely than SGARs to be found in liver tissue. None of the tested muscle tissues contained ARs that were not present in the corresponding liver tissue and all ARs detected in muscle were in lower concentrations than the corresponding liver tissue. This finding is consistent with laboratory findings that ARs concentrate more in the liver than in muscle (Eason 1999). However, the extent to which the ARs were also present in muscle tissue warrants further study, as this is the tissue most likely to be consumed by hunters.

ACKNOWLEDGEMENTS

We thank staff from USDA Wildlife Services and CDFW and Dr. Mourad Gabriel for supplying the samples for this study, and CDFW Large Mammal Advisory Committee for providing laboratory analysis funding.

LITERATURE CITED

Booth, L. H., P. Fisher, V. Heppelthwaite, and C. T. Eason. 2003. Toxicity and residues of brodifacoum in snails and earthworms. DOC Science Internal Series 143, Department of Conservation, Wellington, New Zealand.

Cypher, B. L., S. C. McMillin, T. L. Westall, C. Van Horn Job, R. C. Hosea, B. J. Finlayson. 2014. Rodenticide exposure among endangered kit foxes relative to habitat use in an urban landscape. Cities and the Environment 7(1), Article 8.

Eason, C. T., G. R. G. Wright, L. M. Milne, and G. A Morriss. 2001. Laboratory and field studies of brodifacoum residues in relation to risk of exposure to wildlife and people. Science for Conservation 177 B.:11-23.

Eason, C. T. , L. Milne, M. Potts, G. Morriss, G. R. G. Wright, and O. R.W. Sutherland. 1999. Secondary and tertiary poisoning risks associated with brodifacoum. New Zealand Journal of Ecology 23:219-224.

Elliott, J. S. Hindmarch, C. A. Albert, J. Emery, P. Mineau, F. Maisonneuve. 2013. Exposure pathways of anticoagulant rodenticides to nontarget wildlife. Environmental Monitoring and Assessment 186(2):895-906.

Erickson, W., and D. Urban. 2004. Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. Office of Pesticide Programs, USEPA, Washington, D.C.

Fisher, P., C. O'Connor, G. Wright, and C. T. Easton. 2003. Persistence of four anticoagulant rodenticides in livers of laboratory rats. New Zealand Department of Conservation. DOC Science Internal Series 139, Wellington, New Zealand.

Gabriel, M. W., G. Wengert, J. M. Higley, S. Krogan, W. Sargent, and D. L. Clifford. 2013. Silent forests? Rodenticides on illegal marijuana crops harm wildlife. The Wildlife Professional 7(1):46-50.

Gabriel, M. W., L. W. Woods, R. Poppenga, R. Sweitzer, C. Thompson, S. M. Matthews, J. M. Higley, S. M. Keller, K. Purcell, R. H. Barrett, G. M. Wengert, B. N. Sacks, D. L. Clifford. 2012. Anticoagulant rodenticides on our public and community lands: spatial distribution of exposure and poisoning of a rare forest carnivore. PLoS One 7:340163.

Hosea, R. C. 2000. Exposure of non-target wildlife to anticoagulant rodenticides in California. Proceedings of the Vertebrate Pest Conference 19:236-244.

Kelly, T. R., R. H. Poppenga, L. A. Woods, Y. Z. Hernandez, W. M. Boyce, F. J. Samaniego, S. G. Torres, C. K. Johnson. 2014. Causes of mortality and unintentional poisoning in predatory and scavenging birds in California. Vet Record Open 2014(9):e000028.

Lima, L. L., and T. P. Salmon. 2010. Assessing some potential environmental impacts from agricultural anticoagulant uses. Proceedings of the Vertebrate Pest Conference 24:199-203.

Riley, S. P. D., C. Bromley, R. H. Poppenga, R. A. Uzal, L Whited, and R. M. Sauvajot. 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. Journal of Wildlife Management 71(6):1874-1884.

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

Stone, W. B., J. C. Okoniewski, and J. R. Stedelin. 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. Journal of Wildlife Diseases 35:187-193

.