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Changes in Detected Anticoagulant Rodenticide Exposure in Barn Owls (*Tyto alba*) in Kentucky, USA, in 2012–16

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ABSTRACT: Anticoagulant rodenticides (ARs) are widely used across North America to control rodent infestations but may cause direct mortality or nonlethal effects when secondarily consumed by raptors. Barn Owls (*Tyto alba*) are at high risk for secondary consumption because they specialize in rodent prey and often live in human-made structures. We investigated the exposure of Barn Owls in Kentucky, US, to ARs and to dicoumarol, an anticoagulant compound naturally found in certain moldy forages. We tested the liver tissue of 48 Barn Owl carcasses collected during 2012–16. We confirmed exposure to one or more ARs in 33% of the birds examined and detected dicoumarol in 13% of the samples. Rodenticides detected included brodifacoum, coumachlor, and bromadiolone. The prevalence of detected exposure to brodifacoum for after-hatch-year birds (65%) was significantly (P=0.012) higher than hatch-year birds (22%). Brodifacoum was the most commonly detected AR, found in 88% of AR-positive birds. The pesticide registration for this chemical in the US was canceled in 2015 for general consumer products, which likely resulted in a decreasing rate of detected exposure to brodifacoum during our study. We present these results as an example of secondary exposure rates during a period when a pesticide has been restricted and then removed from the consumer market.

Key words: Anticoagulant rodenticide, Barn Owl, brodifacoum, bromadiolone, dicoumarol, raptor, secondary exposure, *Tyto alba*.

The Barn Owl (*Tyto alba*) is a nocturnal raptor found in open habitats where it preys primarily on small mammals. Severe Barn Owl declines have been recorded in the midwestern US (Marti et al. 2005) and due to local conservation concern, the Kentucky Department of Fish and Wildlife Resources started a population monitoring program for the species in 2010. Dead Barn Owls were collected to determine potential threats to the species.

Anticoagulant rodenticides (ARs) are commonly used across North America to control rodent infestations (Eason et al. 2010). These pesticides can cause direct mortality in raptors when secondarily consumed by disrupting the vitamin K cycle, essential for blood clotting (Kelly et al. 2014). Possible nonlethal effects of ARs have also been documented in raptors (Lemus et al. 2011), including factors affecting productivity (Martínez-Padilla et al. 2016). The Barn Owl almost exclusively feeds on rodents, making this issue a particular concern for this species (Newton et al. 1990). Furthermore, the tendency of Barn Owls to reside and forage in and near human-made structures poses an unusual risk for exposure to ARs authorized for indoor use.

In 2008, the US Environmental Protection Agency published a Risk Mitigation Decision that set forth restrictions on the distribution, sale, and application of brodifacoum and three other ARs (USEPA 2008). Restrictions on packaging, outdoor use, and marketing to general consumers went into effect 4 June 2011, but compliance was slow, and brodifacoum was still widely available for some time after this date. Due to continuing concerns about risks to wildlife, pets, and children, approvals of general consumer products containing brodifacoum, bromadiolone, difenacoum, and difethialone were later canceled. Distribution of these products ended 31 March 2015, and retailers were allowed to sell off stock after that date. Currently, selected brodifacoum and bromadiolone products are still available for use by licensed pesticide applicators (USEPA 2017).

Several studies have documented the exposure of raptors and other wildlife to brodifacoum prior to the 2011 restrictions (Murray

TABLE 1. Minimum levels of detection and quantitation, hepatic detections, and range of concentrations for anticoagulant rodenticides and dicoumarol in Barn Owls (*Tyto alba*) in Kentucky, USA, 2012–16. Dicoumarol is a naturally occurring anticoagulant compound.

Compound	Minimum level		No.		Range of concentrations (μg/kg)[b]
	Detection (μg/kg)	Quantitation (μg/kg)	Trace detections[a]	Quantifiable concentrations	
Brodifacoum	10	20	9	5	24–50
Bromadiolone	20	30	5	1	172
Chlorophacinone	10	40	0	0	—
Coumachlor	0.75	30	2	0	—
Difethialone	10	50	0	0	—
Diphacinone	25	40	0	0	—
Warfarin	0.75	30	0	0	—
Dicoumarol	10	30	6	0	—

[a] Trace detections are concentrations greater than the minimum level of detection and less than the minimum level of quantitation.
[b] — = no concentrations were quantified.

2011; Kelly et al. 2014), but few researchers have examined exposure in recent years. The purpose of our study was to investigate the exposure of Barn Owls in Kentucky to ARs and to the naturally occurring anticoagulant compound, dicoumarol. Dicoumarol is typically not included in standard AR screening. However, we screened for this compound because we thought that the habits of a Barn Owl might lead to dicoumarol exposure. We report findings for ARs and dicoumarol, although we focus on brodifacoum, because our monitoring effort was concurrent with the restriction and cessation of its distribution in the general consumer market.

Barn Owls were collected in Kentucky, US, between January 2012 and December 2016 under a US Fish and Wildlife Service permit (MB036515-0). Most specimens were collected on roadsides, from wildlife rehabilitators, or were reported by the public. All specimens were stored at –20 C until preparation for analysis. We classified the age of each bird as hatch year or after hatch year, on the basis of plumage (Pyle 1997). We also determined the sex for each individual by using the internal gonads observed during necropsy. Specimens too decomposed for tissue collection were excluded from the analysis.

The same pathologist (L.M.C.) performed a complete necropsy on each owl, including a gross and histologic examination of all tissues. Sections of liver from each bird were collected for anticoagulant compound analysis. Liver concentrations of warfarin, coumachlor, diphacinone, dicoumarol, chlorophacinone, bromadiolone, brodifacoum, and difethialone were determined on a wet weight basis, as described by Smith et al. (2017). Briefly, the liver was homogenized and extracted in 10% (v/v) methanol in acetonitrile in a ratio of 1-g sample to 6 mL of extraction solvent. Matrix coextractants were removed from solutions by using dispersive solid-phase extraction; extracts were then concentrated by using evaporative reconstitution in a solution compatible with ultrahigh-performance liquid chromatography–tandem mass spectrometry (Ultimate 3000/TSQ Quantum Access Max, Thermo Scientific, Waltham, Massachusetts, USA). Minimum levels of detection and quantitation are described in Table 1. We considered any detectable amount (including trace amounts) of an anticoagulant compound as evidence of exposure. Trace amounts were defined as concentrations greater than the minimum level of detection but less than the minimum level of quantification.

We conducted all statistical analysis in program R (version 3.3.3; R Development Core Team 2017). We used Fisher exact tests (FET; two tailed, package stats; R Develop-

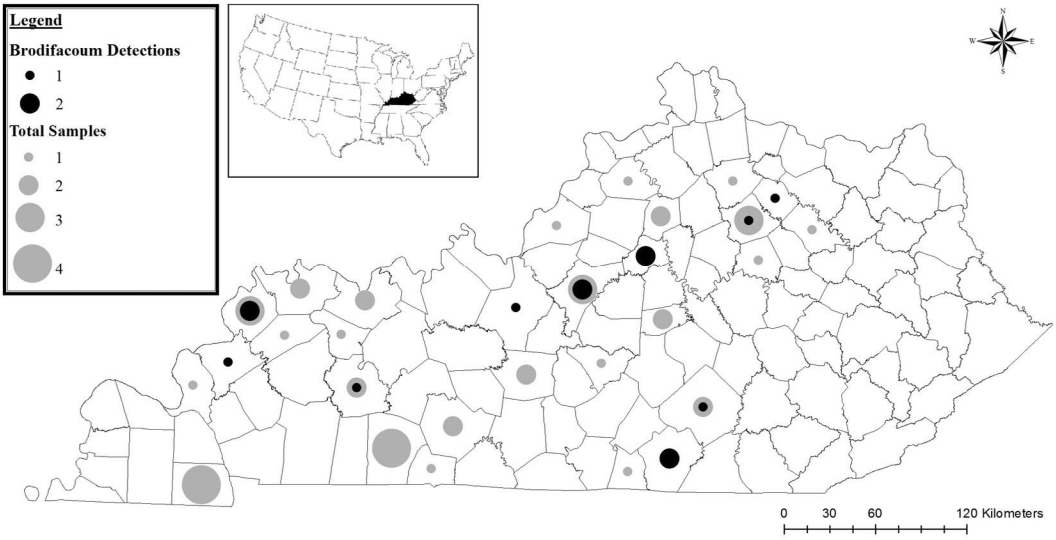

FIGURE 1. Barn Owl (*Tyto alba*) collection locations and proportion of samples positive for brodifacoum in Kentucky, 2012–16. Inset map shows location of Kentucky in the USA.

ment Core Team 2017) to determine if brodifacoum exposure differed among sex or age groups. We then used a generalized linear model to analyze the relationship between brodifacoum exposure and collection year (linear regression package stats; R Development Core Team 2017). The response variable in the single model was the presence or absence of any detectable amount of brodifacoum in each specimen and was modeled with a binomial error distribution and logit link. The predictor variable was collection year.

A total of 51 owls were collected, of which 48 were screened for ARs and dicoumarol. Three were too decomposed for necropsy. We tested one bird in 2012, 10 in 2013, 10 in 2014, 18 in 2015, and nine in 2016. The owls came from the central and western part of the state, which corresponded to the known range of this species in Kentucky (Fig. 1). We identified 46% (22/48) of the owls as female, 38% (18/48) as males, and 17% (8/48) were undetermined sex. Of the owls sampled, 56% (27/48) were hatch-year birds, 42% (20/48) were after hatch year, and one owl was of unknown age. The birds died from various causes but most commonly from trauma (46%, 22/48) and starvation (21%, 10/48). Anticoagulant rodenticide poisoning was not suspected as the primary cause of death for any of the birds, and none of the birds exhibited evidence of an underlying coagulopathy.

Over the course of the entire study, 33% (16/48) of the Barn Owls sampled had residues of at least one AR in their liver. Anticoagulant rodenticides detected included brodifacoum, coumachlor, and bromadiolone. Brodifacoum was the most commonly detected AR, found in 88% (14/16) of AR-positive birds. The overall concentrations of brodifacoum in liver tissues ranged from trace in 9/14 birds to quantifiable levels up to 50 μg/kg in 5/14 birds (Table 1). Bromadiolone was detected in 38% (6/16) of AR-positive birds. Most detections for this compound were for trace amounts (5/6), but we did quantify the concentration for one sample (172 μg/kg, June 2016). Thirty-one percent (5/16) of AR-positive birds tested positive for exposure to more than one AR. Six of the 48 samples tested positive for trace amounts of naturally occurring dicoumarol, with three in the absence of ARs and three in association with ARs.

The prevalence of detected exposure to brodifacoum was not significantly different between males and females (FET, P=0.742). However, the prevalence of detected exposure

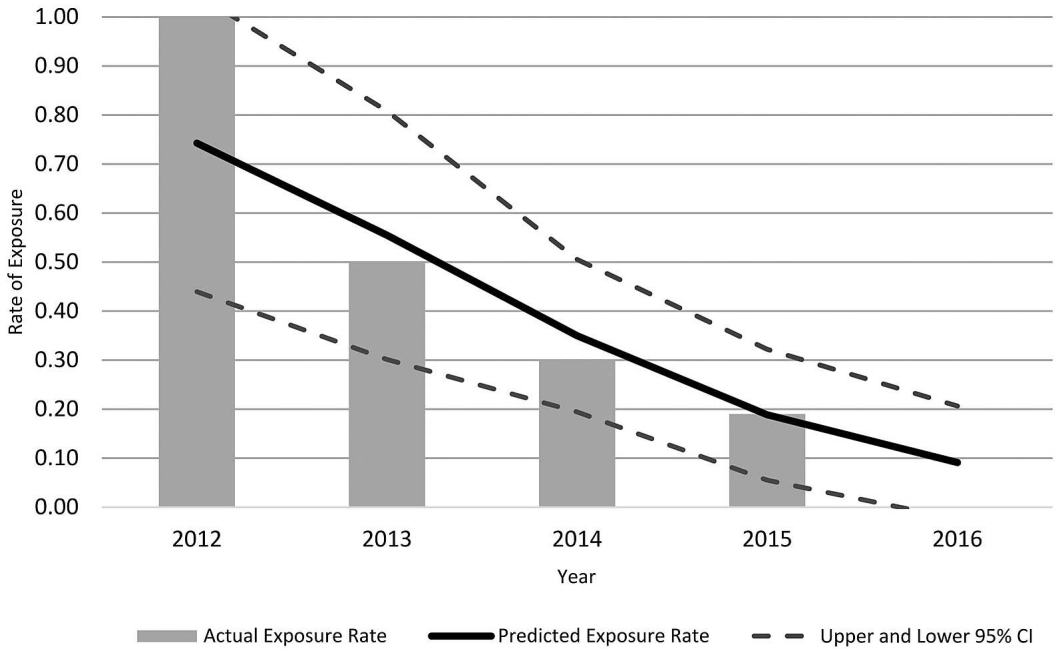

FIGURE 2. Detected exposure and predicted exposure detection over time (year) to brodifacoum in dead Barn Owls (*Tyto alba*) collected in Kentucky, USA, between 2012 and 2016. Solid line is derived from estimates from a generalized liner model by using a logit link (presence or absence of brodifacoum on the basis of year). Dashed lines represent the upper and lower 95% confidence intervals (CI) for the predicted exposure rate.

to brodifacoum for after-hatch-year birds was significantly higher than hatch-year birds (FET, odds ratio=5.5147; P=0.012, 95% confidence interval: 1.229–30.248). Collection year was negatively associated with brodifacoum detection when fitted with the generalized linear model, and brodifacoum detections significantly declined as calendar year increased (linear regression, estimate=–0.840; SE=0.338, P=0.013; Fig. 2).

Despite the 2011 restrictions on brodifacoum, a substantial proportion of the Barn Owls collected for our study were still being exposed to the pesticide until the cancellation of all general consumer products in March 2015. Few published studies exist on secondary exposure to brodifacoum since the 2011 requirements, but Justice-Allen and Loyd (2017) also found brodifacoum exposure in Western Burrowing Owls (*Athene cunicularia hypugaea*) during 2013–15 in Arizona, US. These findings demonstrated that the 2011 restrictions were likely not sufficient to alleviate the threat brodifacoum posed to birds of prey.

Anticoagulant rodenticides can be progressively accumulated in the liver (Newton et al. 1990); therefore, age may have a significant effect on rodenticide exposure. As expected, we found the prevalence of AR detection was significantly higher in after-hatch-year birds than in hatch-year birds. Our results were similar to a study in Canada that found the proportion of juvenile Barn Owls exposed to bromadiolone to be significantly lower than the proportion of adults (Huang et al. 2016). Higher exposure in adults may result from accumulation from repeated exposure or could simply be due to a longer lifetime, allowing more opportunities for exposure to poisoned prey.

Although not the focus of our study, we screened for dicoumarol to fully explain potential coagulopathies and investigate the prevalence of exposure. We were surprised to detect dicoumarol in 13% (6/48) of the owls (Table 1). Our identification criteria for this

compound included two product ions, expected retention times and appropriate product ion ratios. Thus, we were confident that we detected true exposure to dicoumarol. Our detection of dicoumarol is interesting because it is not currently in use as an AR. Instead, this substance is found naturally in certain moldy forages (e.g., sweet clover, *Melilotus* spp.). There is little research on the exposure of wildlife to dicoumarol, especially secondary exposure in predators. Exposure to this anticoagulant is better documented in livestock and is known to cause the hemorrhagic disease, sweet clover poisoning (Lefebvre et al. 2017). Barn Owls likely become exposed to dicoumarol after eating prey that have consumed moldy hay. The tendency of Barn Owls to nest and roost in hay barns probably presents an increased risk of dicoumarol exposure, in comparison to other raptor species. More study is needed to understand the effects dicoumarol exposure may have on Barn Owls and other wildlife.

We did not detect brodifacoum in any samples collected in 2016 (Fig. 2), but we do not assume brodifacoum exposure became a nonissue in the latter part of our study. Due to the rarity of Barn Owls in Kentucky, our sample size was relatively small, and the number of owls examined no doubt influenced our ability to detect reduced rates of exposure (Shore et al. 2014). Nonetheless, our predicted exposure rate over time (Fig. 2) indicated some amount of secondary exposure to brodifacoum may persist into the future. This continued but reduced rate of expected exposure to restricted ARs is substantiated by our detection of bromadiolone (172 μg/kg) after restrictions began in June 2016. Because Barn Owls often use structures at commercial facilities and agricultural compounds for nesting and foraging, they continue to be at some risk for exposure to ARs. Selected brodifacoum and bromadiolone products are still available for use by licensed pesticide applicators and for agricultural use, indoors or outdoors within 30 m of human-made structures (USEPA 2017). Still, based on our results, we assumed the risk of exposure to brodifacoum has been reduced by the recent restrictions.

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