What Do We Need to Know to Assess Individual and Population-level Effects on Wildlife from Anticoagulant Rodenticides?

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ABSTRACT: Anticoagulant rodenticides have been detected in many species of wildlife worldwide; yet the origins, exposure pathways, and effects of this exposure are not well understood. Furthermore, to accurately characterize the risks from rodenticide use, information is needed on what proportion of populations are being exposed, what proportion of the exposed individuals are affected, and in what ways. The relationship between anticoagulant rodenticide concentrations found in wildlife and the rate of mortality or illness is the subject of much current research. Residue levels observed in liver and whole body analyses vary, and overlap extensively among apparently healthy asymptomatic individuals and sublethal and lethal cases. Results from laboratory studies also show there can be wide variability in lethal and sublethal effects among and within taxonomic groups. Correlating the sublethal and reproductive effects observed in laboratory studies with realistic exposure scenarios and effects in the wild is needed to improve risk assessments. For species with limited numbers/declining populations, a critical question is whether the rodenticide exposure documented in individual animals inhibit population growth or contribute to population declines by lowering survival and reproductive success. This information is essential to the regulatory agencies that must weigh the risks and benefits of rodenticide uses and identify restrictions that are effective in reducing risks to wildlife. A primary objective of this symposium was to facilitate communication between regulators and researchers. Current research on many of these topics was presented, and was followed by discussions on how to improve our understanding of what factors lead to wildlife exposure and improve our ability to assess the effects of exposure on individuals and populations. A collaborative approach will be developed to design studies that provide regulatory and wildlife management agencies with additional science on which to base their decisions.

KEY WORDS: anticoagulant rodenticides, lethal effects, residues, sublethal effects, wildlife

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INTRODUCTION

Anticoagulant rodenticides are widely used, toxic to a broad range of taxa, and are persistent in many organisms. A symposium on nontarget mortality and the environmental fate of rodenticides was held at the 24th Vertebrate Pest Conference in 2010 (Eisemann et al. 2010). Presenters assessed the risks from conservation and agricultural rodenticide uses, examined specific incidents of nontarget mortality, and discussed measures and policies that could reduce the risk of nontarget exposure. Since then, despite mitigation measures implemented by the EPA and the State of California to reduce exposure, much of the current research on the environmental effects of rodenticides has continued to document its occurrence in wildlife. The lack of effectiveness of mitigation measures shows the need for more information on how exposure occurs. Furthermore, existing methods for conducting risk assessments for the anticoagulant rodenticides have been inadequate at predicting the occurrence of physiological effects in individuals in wild populations. Speakers for the 28th Vertebrate Pest Conference's Symposium "Anticoagulant Residues in Wildlife" were selected to provide an update and overview of this topic. Papers were grouped into three lines of inquiry:

1. Presence/prevalence of anticoagulant rodenticides in wildlife;
2. Pathways of anticoagulant rodenticide exposure in wildlife;
3. The impacts of rodenticide exposure on wildlife.

The presentation of multiple papers within these subject areas provided a broader context within which to evaluate the information from each study. The ranges of approaches and methods used how data differed or were in agreement, and the conclusions drawn, improved understanding of the issues while also reinforcing the need to accelerate progress in identifying and addressing knowledge gaps. This paper briefly summarizes the Symposium's presentations and some relevant literature. The papers themselves should be read, and van den Brink et al. (2018) is the definitive compendium for essential background knowledge for the Symposium.

Presence/Prevalence of Anticoagulant Rodenticides in Wildlife

Awareness of hazards to nontarget wildlife from anticoagulant rodenticides emerged in the 1970s and 1980s (Kaukeinen 1982, Godfrey 1985, Colvin et al. 1988). Subsequent studies largely focused on documenting exposure (Eason and Spurr 1995, Berny 2007, Albert et al. 2010). Anticoagulant rodenticides have been found in a broad range of taxa in a number of countries, although there appears to be a geographic bias, with most studies

being conducted in North America, Europe, and New Zealand. Nonetheless, wherever people have tested animals for rodenticide residues, they have found them (Winters et al. 2010, Sánchez-Barbudo et al. 2012, Langford et al. 2013). There have been detections of anticoagulant rodenticides in marine species (Pain et al. 2000, Primus et al. 2005, Pitt et al. 2015), invertebrates (Spurr and Drew 1999, Bowie and Ross 2006, Elliott et al. 2014) and in reptiles (Pitt et al. 2015, Rueda et al. 2016). Anticoagulant residues have been detected in a number of bird species, including raptors (Newton et al. 1990, Stone et al. 2003, Murray 2011), passerines (Pryde et al. 2013, Elliott et al. 2014), waterfowl (McMillin and Finlayson 2010), and game birds (Ruder et al. 2011). They have also been detected in a wide range of mammals. These detections are not just limited to predators (Shore et al. 1999, Riley et al. 2007); they have also been detected in insectivorous (Dowding et al. 2010) and herbivorous mammals (Eason et al. 2001).

The first group of papers in the Symposium presented information on the presence and prevalence of exposure by taxa, including raptors (Murray 2018) and other bird species (Vyas 2018); game animals (McMillin et al. 2018) and mountain lions (*Puma concolor*) (Rudd et al. 2018) in California; and domestic animals and wildlife in France. Animals sampled included live, injured raptors brought in to a wildlife clinic (Murray 2018); opportunistically-found carcasses and nuisance animals taken under depredation permits submitted to the California Department of Fish and Wildlife (CDFW) (McMillin et al. 2018, Rudd et al. 2018); carcasses and poison hotline call information submitted to a national database in France; and a compilation of data from published incident reports (Vyas 2018). The prevalence of rodenticide exposure ranged from none in deer (*Odocoileus hemionus*) in California, to very high percentages of the individuals tested for mountain lions in California and raptors in Massachusetts. Presenters noted the limitations in their methods and how this impacted their conclusions, particularly when assessing the effectiveness of regulations in California and the European Union.

One of the main limitations with interpreting exposure results is that they must be placed in the context of populations. By not reporting the number of animals tested as a proportion of the total population, studies of detections in individual animals do not provide a population-level assessment of exposure. Anticoagulant rodenticide residues are often detected through studies on threatened or endangered species, charismatic megafauna, or on spotlight species, especially raptors. These studies are not random samples of populations; the methods by which individuals are selected for rodenticide testing introduce inaccuracies due to multiple and contradictory factors. Testing only dead and symptomatic individuals, but not those that appear to be healthy, does not measure the actual proportion of the population that is exposed. This type of nonrandom sampling design based on the greater likelihood of detection of symptomatic individuals also does not accurately assess the proportion of exposed animals that are affected by the exposure, because animals unaffected by exposure are not included at all. If sublethal effects are present, they may be difficult to detect and therefore these animals are not selected for testing and are also not included in the results. Animals that have succumbed to rodenticide intoxication are also underrepresented because they may not be discovered. Carcass detection studies have found that even when searches are performed on carcasses known to exist (e.g., placed by a researcher for study), a percentage will never be found due to scavenging, location in remote and inaccessible areas, or size or coloration that renders the carcass inconspicuous (Vyas 1999, Elliott et al. 2008). Public reporting of wildlife mortalities in general is limited both by detection of carcasses as well as uncertainty as to whether the incident should be reported and to whom it should be reported, procrastination, and indifference (Vyas 1999).

Exposure Pathways

Detailed information about rodenticide exposure pathways is essential for designing effective mitigation measures. Modifications to how rodenticides are applied are unlikely to be successful at reducing nontarget exposure if it is not understood how rodenticides travel from the point of application to nontarget species. Studies examining the initial stages of rodenticide transfer from known agricultural or commensal application sources have documented the widespread transfer of rodenticides into both target and nontarget species in the surrounding areas (Silberhorn et al. 2003, Tosh et al. 2012, Vyas et al. 2013, Elliott et al. 2014, Geduhn et al. 2014). The bait in these studies was applied according to legal methods (except as noted in Tosh et al. 2012), in many cases by the researchers themselves, yet the rodenticides were still detected in a wide range of nontarget taxa, from invertebrates to small mammals to passerines and raptors. This clearly demonstrates that the processes by which the rodenticide travels beyond the point of application are outside of the control of the applicator. It is therefore not surprising that mitigation measures based on the assumption that professional applicators will apply rodenticides more safely (e.g., US EPA 2008, CDPR 2013) have not resulted in a measurable decline in wildlife exposures.

Wildlife are exposed to anticoagulant rodenticides through a number of pathways, which vary considerably in their complexity. Constructing an exposure pathway requires accurate information about the source of the rodenticide, each species' diet and foraging behavior, and the true prevalence of exposure within the populations. Rodenticides are applied in agricultural and field sites (e.g., fallow cropland, around crop borders, in and around orchards and tree nurseries, rangeland, dikes, parks, and landscaping); and in commensal sites (in and around buildings) in urban, suburban, and rural areas. In many countries, specific active ingredients can only be legally applied for specific sites, uses, and against particular species. First generation anticoagulant rodenticides (FGARs) are mostly used to control field rodents in agriculture and sites away from human habitation, whereas the SGARs are limited to application in and around structures to control commensal rats and mice, with the exception of bromadiolone, which has field uses outside of the U.S. The FGARs are also used to control commensal rodents in and around structures.

From the point of application, exposure to the rodenticide can be primary, secondary, tertiary, or at further levels. Primary exposure is defined as the direct consumption of the rodenticide; secondary exposure results from the ingestion of prey that has fed on the rodenticide; tertiary exposure occurs when an organism consumes prey that has predated on an organism that has been exposed, and so on. An individual animal can be exposed at more than one level and from different rodenticide sources over a period of time. Residues of multiple anticoagulant rodenticides, including both FGARs and SGARs, are often detected in individuals.

For some nontarget wildlife species, there does not appear to be a connection with the target species and/or the site or method of bait application. Some primarily exposed species are not known to enter bait stations, or otherwise have had no access to bait. Some species that are secondarily exposed through predation on rodents, or exposed at the tertiary level or further, such as mesopredators like coyotes (*Canis latrans*) or apex predators like mountain lions, do not prey on the target species, leaving their route of exposure unknown. The original source(s) of the rodenticide are often unknown; the nearest known source may be distant, outside of the species' habitat, and/or the individual's home range (Berny 2007). The delayed toxicity of anticoagulant rodenticides and their persistence within tissues can result in contaminated rodents being found within and adjacent to the treated area weeks or months after bait application (Sage et al. 2008, Tosh et al. 2012, Geduhn et al. 2014). After brodifacoum applications for island eradications of introduced rodent species, the long half-life of brodifacoum in tissues has resulted in it cycling through food webs in the ecosystems for months or years (Ebbert and Burek-Huntington 2010, Pitt et al. 2015, Rueda et al. 2016, Siers et al. 2016).

One Symposium paper explored aspects of exposure pathways for raptors: Hindmarch (2018) proposed typical traits of affected species based on a study of raptors in a range of habitats in British Columbia. Hindmarch (2018) also found that exposure pathways vary considerably among species and habitat type. Both presenters suggested that invertebrates and avian species could be significant sources of rodenticide exposure that should be included in the investigation of exposure pathways. No papers on mammalian exposure pathways were submitted for the Symposium.

The few papers submitted on this topic reflect the difficulty in studying exposure pathways. The methods employed are indirect and generally involve working backwards from the exposed species to many potential application sources within a broad area. Scat analyses for anticoagulant rodenticides are an example of an indirect method that provides only limited information due to the low likelihood of detection of the scats themselves and the likelihood of misidentification of the depositing species (Morin et al. 2016). Camera and direct visual observations of nontarget species' interactions with bait or the target species (Vyas 2017, Quinn unpubl. data 2018) provide clear information at the source of the rodenticide application that can be used to modify application methods. Biochemical analytical methods, such as the use of stable isotopes in custom-marked rodenticide baits, could be utilized to trace the rodenticide from a point source through food webs.

A critically important limitation of the current state of knowledge for exposure pathways is that they are qualitative, and therefore unable to predict the likelihood of exposure for individuals, the proportion of a population that is exposed, and the effect on survivorship or other demographics as a result of the exposure. New approaches to detect and quantify the proportion of applied rodenticide that travels through specific routes to each nontarget species are urgently needed, along with more emphasis on developing probabilistic models of exposure and its effects.

Effects of Anticoagulant Rodenticide Exposure

Rodenticide exposure to wildlife is a multi-faceted issue that encompasses more than whether an individual has been exposed. Data on the magnitude of the exposure and what effect(s) the exposure has are necessary to evaluate the consequences of the exposure (e.g., Berny 2007). Research in this area has focused on three lines of inquiry: laboratory studies of toxicity in surrogates species; correlating the levels of anticoagulant residues in tissues with specific toxicological endpoints; and identifying effects other than direct mortality. The four final papers of the Symposium (Horak et al. 2018, Rattner et al. 2018, Serieys 2018) covered these topics.

The toxicity of the anticoagulant compounds has been assessed in laboratory studies for a small number of species. These values are of limited utility for determining the effects of exposure on wildlife because susceptibility to the anticoagulants varies substantially between individuals and species (Erickson and Urban 2004). Such studies have also been criticized for being conducted under conditions that result in unrealistic toxicity estimates (Vyas and Rattner 2012). The U.S. Fish and Wildlife Service has called for a more comprehensive approach to assessing the effects of pesticides on endangered species than has been used based on the single toxicological endpoint of mortality (Golden et al. 2011).

Concurrent with awareness that exposure to nontarget wildlife was occurring, early research on the effects of exposure focused on observing symptoms of toxicosis in laboratory studies to determine the effects of exposure, including mortality. Raptors and mammals were fed rodents or other animal tissues containing rodenticides under controlled conditions, but the dose was not measured (e.g., Evans and Ward 1967, Savarie et al. 1979, Mendenhall and Pank 1980). Symptoms documented in these studies, and in the veterinary and medical literature, include lethargy, anorexia, ataxia, anemia, lameness or immobility due to bleeding in the joints, and difficulty breathing (DuVall et al. 1989, Merola 2002, Spahr et al. 2007, Murray and Tseng 2008, Valchev et al. 2008). Work by Rattner et al. on captive kestrels (*Falco sparverius*) (Rattner et al. 2011) and Eastern screech-owls (*Megascops asio*) (Rattner et al. 2012, Rattner et al. 2014a) examined the pharmacokinetics of first generation anticoagulant exposure and developed toxicity reference values for a range of sublethal effects, including coagulopathy and hemorrhaging. Their most recent research, presented at the Symposium, investigated the sublethal effects of

sequential exposures to a FGAR (chlorophacinone) and a SGAR (brodifacoum).

Exposure to anticoagulant rodenticides is confirmed by chemical analysis of the liver, other body tissues, blood, or the whole carcass, for the specific anticoagulant compound (Vandenbroucke et al. 2008, Rattner et al. 2014b). Given the low concentrations of the rodenticides in the baits (25 - 50 parts per million (ppm)), residue concentrations detected in exposed individuals are at similarly low levels, or even in the parts per billion level (ppb or µg/kg) (Erickson and Urban 2004, Dowding et al. 2010). Rodenticide levels in blood and tissues are determined by a multitude of factors, including the concentration in the bait (Kaukeinen 1982, Merson et al. 1984), the amount of bait consumed, the length of time the individual was exposed (single feeding or chronic (dietary)), the time elapsed since the last exposure (Merson et al. 1984), the half-life of the compound in the specific biological matrix, and the rate at which an individual metabolizes and excretes the compound (Erickson and Urban 2004). Residue values cannot be used to determine the magnitude of the dose an individual has been exposed to since they vary widely even between individuals exposed to the same dose (Fisher 2006, Rattner et al. 2014a). Due to these factors, residue values from individuals exposed to the same rodenticide application will vary (Merson et al. 1984, Primus et al. 2001, Ebbert and Burek-Huntington 2010, Vyas et al. 2012).

Furthermore, the detection and quantitation of the anticoagulant rodenticides in biological matrices, such as blood and liver, may not be comparable between studies. There is considerable variation in the techniques used to recover rodenticides from sample matrices, as well as in the chemical analysis methods used to detect them (Goldade et al. 1998, Marek and Koskinen 2007, Vandenbroucke et al. 2008, Thomas et al. 2011). One study found that the chemical analysis method could significantly underestimate the prevalence of SGARs in wildlife (Dowding et al. 2010). To ensure that effects are conclusively attributable to rodenticide exposure, other toxic compounds (e.g., lead, mercury, selenium, organophosphates and other pesticides) and diseases (e.g., West Nile virus, avian influenza) should be tested for (Berny and Gaillet 2008, Kelly et al. 2014, Gabriel et al. 2015, Siers et al. 2016). The importance of conducting a thorough investigation to rule out other causes of mortality was stressed during the 2010 Symposium (Ebbert and Burek-Huntington 2010).

Studies attempting to correlate levels of anticoagulant exposure with effects have reported wide variability in lethal and sublethal effects among and within taxonomic groups, and no consistent trend has been identified (Erickson and Urban 2004, Rattner et al. 2014c, Murray 2017). For example, no correlations between residue level and mortality or symptoms of toxicosis were found in several studies on wild raptors environmentally-exposed to rodenticides (Albert et al. 2010, Murray 2011), whereas laboratory studies with controlled doses of diphacinone and chlorophacinone in kestrels did find correlations between mortality or symptoms of toxicosis and liver residue levels (Rattner et al. 2011, Rattner et al. 2015). A probabilistic model using published data of liver SGAR concentrations from 270 individuals of four raptor species (barn owl *Tyto alba*, barred owl *Strix varia*, great horned owl *Bubo virginianus*, and red-tailed hawk *Buteo jamaicensis*) estimated probabilities of toxicosis as a function of summed quantities of each anticoagulant liver residue value, for a total exposure per individual (Thomas et al. 2011). They found significant differences between species in the residue values at which symptoms occurred. When pooling the data from all four species (69 positive out of the 270 birds), one in twenty birds were predicted to show signs of toxicosis when liver concentrations were 0.02 mg/kg, and one in five were predicted to show signs of toxicosis when liver concentrations reached 0.08 mg/kg. Thomas et al. (2011) note that their results are applicable to the three owl species only. While the probabilities estimated for specific residue values would be helpful in analyzing large data sets for these three owl species, they cannot be used to determine whether an individual owl with a given residue level succumbed to SGAR exposure, nor can they be used to conclude that individuals of other species were fatally exposed.

Because residue concentrations have not been consistently linked to thresholds for which adverse effects are expected to occur across different species, diagnoses using these data must be accompanied by full necropsy results (Berny 2007, Ebbert and Burek-Huntington 2010, Murray 2011). The lethal effects of exposure to anticoagulant rodenticides can often be confirmed by symptoms in necropsy. They generally include evidence of extensive hemorrhage (subcutaneous, intramuscular, pulmonary, visceral, or intracoelomic hemorrhage, pallor of internal organs) without concurrent evidence of corresponding severe trauma (such as fractures, wounds, or ocular injury) (Murray 2011). In individuals with no obvious symptoms, histological examination can detect microhemorrhages (Rattner et al. 2011).

Sublethal effects of anticoagulant exposure other than coagulopathy and hemorrhaging are more difficult to document in wildlife. Sublethal effects observed in laboratory and clinical settings include anorexia, impaired mobility, and difficulty thermoregulating (Savarie et al. 1979, Swift 1998, Murray 2011, Vyas et al. 2014, Rattner and Mastrota 2018). Similar measures for confirmation of anticoagulant rodenticide exposure as the cause of mortality should be undertaken to confirm anticoagulant rodenticide exposure as the cause of a sublethal effect in sick individuals. The presence of an anticoagulant rodenticide in the blood or tissues is not conclusive, and other causes, such as pathogens, other pesticides and anthropogenic contaminants should be tested for.

Few studies have examined physiological effects not directly related to impaired blood clotting. Data from a wild population of bobcats (*Lynx rufus*) was used to identify the mechanisms by which anticoagulants could interfere with various aspects of immune-system function (Serieys et al. 2018) and gene expression (Fraser et al. 2018). However, researchers were unable to produce immunosuppressant effects in domestic cats (*Felis catus*) in a laboratory study with brodifacoum (Kopanke et al. 2018). The inconsistent findings and conclusions from this group of studies highlight the need for significantly more research in this area.

Documentation of the reproductive effects of exposure to anticoagulant rodenticides is rare (Munday and Thompson 2003, Rady et al. 2013). A study of barn owls (*T. alba javanica*) foraging in Malaysian oil palm plots treated with bromadiolone or chlorophacinone observed no effects (Salim et al. 2014). No significant difference in eggshell thickness was found between birds that foraged in the treated plots and those from untreated plots, despite detectable levels of the compounds in the eggs. Similarly, reproductive effects from sublethal exposure to anticoagulant rodenticides are difficult to determine in laboratory studies. Mineau et al. (2005) provides an extensive critique of why the standard laboratory reproductive toxicity tests with captive mallard and bobwhite are not likely to accurately assess the effects of pesticide exposure on wild birds.

Differences in the pharmacokinetic properties of stereoisomers of the SGARs could provide insights into the mechanisms by which ARs cause toxicity. In laboratory studies with rats, the diastereoisomers of each compound have different affinities for binding in the liver, resulting in differences in their liver half-lives. However, the toxicity of the stereoisomers to nontarget species, especially birds, has not yet been evaluated. These preliminary findings have significant implications. If the toxicity is lower for nontarget species, and if the stereoisomers with the lower liver persistence can be produced in greater proportions, the ecotoxicity of the SGARs potentially could be reduced.

CONCLUSIONS

What Are Priorities for Rodenticide Residue Research?

The Symposium illustrates the benefits from the approach of examining a number of studies together and synthesizing the conclusions. By evaluating the three main components of the issue of rodenticide residues in wildlife grouped together, common themes were apparent and a clear path forward of needed research emerged.

These questions recurred through many of the presentations and/or were noted as important during the subsequent discussion:

1. What proportion of a population is being sampled?
2. What proportions of exposed individuals are compromised?
3. How are primarily exposed nontarget species accessing rodenticide baits?
4. How are predators/scavengers being exposed when their diets don't include the target species?
5. How do we identify source locations (point of application)?
6. How do we apply toxicity results from small groups of surrogates in lab studies to wild populations of different species?
7. How should tissue residue values be interpreted?
8. What are the causal mechanisms linking exposure to sublethal effects?
9. What can be done to lower the ecotoxicity of the rodenticides?

As with any stressor on a wildlife species, once identified the next step is to determine the magnitude of its effect. Data quantifying the rate at which rodenticide exposure is occurring within populations, and the proportion of exposed individuals affected, either directly or indirectly, are needed. Testing dead and moribund individuals is an inherently biased sampling design since it only examines a subset of a population while excluding the living portion. Studies should be designed to ensure that: all individuals (live, moribund, and dead) within a population have an equal probability of being selected for rodenticide and other contaminant testing; their health and other potential causes of symptoms are assessed; and sample sizes are robust enough to support statistical analyses. Since this is more challenging for rare and/or difficult to detect species, concurrent sampling of more common surrogate species occupying the same geographic area could be cautiously used to supplement data for the rarer species. A direct measure of the effect of the rodenticide exposure on survivorship and/or reproduction for each individual sampled must be included and compared against unexposed individuals within the population. The results can then be used to calculate the extent of the exposure and draw conclusions regarding the impact it is having on the population.

Among the conclusions from the Symposium were that for particularly important, sensitive and/or rare species, because of the high degree of variation in exposure and effects between individuals, species, and even between populations, as few extrapolations from other species should be used as possible. Finally, just as the concern over the development of resistance to the first generation anticoagulants spurred the development of new compounds to restore efficacy, the concern over the effects on wildlife should result in more efforts to develop rodenticides with lower ecotoxicity.

Other priorities that emerged were research to evaluate current application practices using quantitative measures of efficacy, and to use that information to develop best practices for the use of rodenticides within an Integrated Pest Management framework.

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