

Anticoagulant Rodenticides and Raptors: Recent Findings from New York, 1998–2001

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Anticoagulant rodenticides are currently the principal means of controlling commensal rodents worldwide. These pesticides interfere with vitamin K-mediated synthesis of blood clotting factors in the liver. Once circulating clotting factors are lost by normal attrition over a few days, animals that have ingested anticoagulants are vulnerable to fatal hemorrhage precipitated by minor trauma, exertion, and possibly other factors. Unlike warfarin, the first anticoagulant product introduced in the 1940's, many of the anticoagulant rodenticides presently used show significant physiological persistence (Bachman and Sullivan 1983, Huckle et al. 1988, Parmar et al. 1987). As a result, single exposures may be lethal and multiple sublethal exposures may produce a cumulatively fatal result. Showing high acute toxicity for a variety of species, these contemporary anticoagulants can pose a threat to non-target animals which may be attracted to the bait directly, or which may prey upon or scavenge animals that have ingested the bait. Risks to predators and scavengers appear to be especially high with brodifacoum, a product that was introduced in the early 1980's. Feeding trials with captive owls (Mendenhall and Pank 1980, Newton 1999), a study of radio-marked screech owls (*Otus asio*) around experimentally treated orchards (Hegdall and Colvin 1988), observations of barn owl (*Tyto alba*) mortality in a palm oil plantation (Duckett 1984), assessments of non-target wildlife mortality in New Zealand where brodifacoum is used to control introduced mammals (Eason and Spurr 1995), poisoning of common ravens (*Corvus corax*) during rat control in a seabird colony (Howald et al. 1999), and documentation of anticoagulant-caused wildlife mortality in New York State (USA) (Stone et al. 1999), all attest to the potential for brodifacoum to kill non-target wildlife.

In New York, the documented incidents of wildlife mortality from anticoagulant rodenticides increased markedly in the 1990's. Through 1997, analyses for anticoagulants were largely reserved for cases in which there was marked hemorrhage in the absence of severe trauma. Suspecting that anticoagulant-related coagulopathy was a likely contributing factor to more complex cases, and wishing to gain insight relative to the frequency of exposure for some raptor and vulture species, analyses of liver for anticoagulants were subsequently completed for a broad spectrum of gross post-mortem findings from January 1998 through June 2001 and are presented here.

MATERIALS AND METHODS

Most of the raptors in this report were submitted for diagnostic purposes directly or indirectly by the general public. Beginning in the late summer of 1999 the routine submission of birds to our wildlife pathology laboratory was augmented by a surveillance program (directed by the New York State Department of Health and implemented by cooperating county and municipal health agencies) related to an outbreak of West Nile Virus in the greater New York City region and, eventually, the remainder of the state

Complete necropsies were performed on all specimens. Livers were collected and stored at -25°C. Analyses for anticoagulants were completed at the Illinois Animal Disease Laboratory (Centralia, Illinois, USA) using a high-performance liquid chromatography screening procedure based on modifications of Chalermchaikit et al. (1993). Following an acetone extraction of 2g samples of liver and a solid-phase cleanup using Florisil and C-18 Sep Pak cartridges (Waters Corporation, Taunton, Massachusetts) in tandem, identification and quantitation of 12 different anticoagulants were achieved by reverse-phase separation using both UV and fluorescence detectors (Shimadzu models SPD-M10AVP and RF-10AXL; Shimadzu Scientific Instruments, Inc., Columbia, Maryland, USA). Additional sensitivity and confirmation were attained when necessary with an ion-pairing method (Hunter 1985), particularly for indandione compounds. Wet-basis detection limits for this procedure were 0.003 ug/g for brodifacoum and bromadiolone; 0.008 ug/g for warfarin, fumarin, and coumachlor; 0.01 ug/g for difenacoum; 0.05 ug/g for chlorophacinone, diphacinone, and coumatetralyl; and 0.15 ug/g for pindone, valone, and difethialone.

Subsets of analytical data were compared with single factor ANOVA, t-tests, and tests for proportions (Snedecor 1980).

RESULTS AND DISCUSSION

Anticoagulants were detected in 49% of 265 raptors (Table 1). Detections were recorded in 12 species, and were especially frequent (81%, n = 53) in great horned owls (*Bubo virginianus*). Based on finding of acute lethal blood loss in the absence of severe injury or other non-toxicological hemorrhagic factors, anticoagulant facilitated hemorrhage was considered the cause of death in 9 cases (14.6% of positive cases, 7.2% overall). Anticoagulants were also strongly considered a possible mortality factor in at least nine additional cases. Anticoagulants were detected in 43% of the remaining birds. Negative analytical findings were recorded in seven cases in which anticoagulants were initially suspected as probable or possible mortality factors.

Table 1. Frequency of detection of anticoagulant (AC) rodenticides in livers of raptors and vultures in New York State (U.S.A), 1 Jan 1998 - 31 Dec 2001.

Species	n	detections	AC mortalities ^a
Bald eagle (<i>Haliaeetus leucocephalus</i>)	5	1	0
Sharp-shinned hawk (<i>Accipiter striatus</i>)	11	1	1
Cooper's hawk (<i>Accipiter cooperii</i>)	50	18	1
Northern goshawk (<i>Accipiter gentilis</i>)	1	0	0
Red-tailed hawk (<i>Buteo jamaicensis</i>)	78	45	12
Broad-winged hawk (<i>Buteo platypterus</i>)	11	0	0
Rough-legged hawk (<i>Buteo lagopus</i>)	1	0	0
Peregrine falcon (<i>Falco peregrinus</i>)	2	1	1
Merlin (<i>Falco columbarius</i>)	1	0	0
Golden eagle (<i>Aquila chrysaetos</i>)	1	1	0
Screech owl (<i>Otus asio</i>)	22	10	1
Great horned owl (<i>Bubo virginianus</i>)	53	43	9
Barred owl (<i>Strix varia</i>)	13	3	1
Long-eared owl (<i>Asio otus</i>)	7	2	1
Short-eared owl (<i>Asio flammeus</i>)	1	0	0
Saw-whet owl (<i>Aegolius acadicus</i>)	3	1	0
Snowy owl (<i>Nyctea scandiaca</i>)	2	0	0
Turkey vulture (<i>Cathartes aura</i>)	2	2	1
Black vulture (<i>Coragyps atratus</i>)	1	0	0

^aNumber of cases in which anticoagulants were considered as principal (19) or probable (9) mortality factors based on necropsy findings and analytical results.

Thirty-nine percent of the positive cases originated from the greater New York City/Long Island region. Most other regions of the state were represented in the remaining positives with the notable exception of the Adirondack and Catskill Mountains. This distribution of cases generally reflects the overall geographic pattern of submissions for this period. Urban and suburban origins were more common than rural origins, at least in part because of a higher probability of discovery in densely populated areas. Overall frequencies of detection did not differ significantly relative to geographic origin.

Brodifacoum was detected in 84% of the positive cases, and was the sole anticoagulant detected in all but one of the 28 cases in which anticoagulants were suspected as mortality factors. Bromadiolone was detected in 22% of cases but strongly suspected in the proximal cause of death in only two instances. Brodifacoum and bromadiolone were found in combination in 15 birds. Also

detected, but not definitively implicated in mortalities were diphacinone (3 cases), warfarin (2), and chlorophacinone (1).

Among positive cases, brodifacoum levels averaged 0.18 ug/g (range = 0.005 - 1.28 ug/g). The mean level in cases in which brodifacoum was considered a mortality factor (0.36 ug/g, range = 0.03 - 1.28) was greater ($P = 0.00005$) than the mean for the other cases with brodifacoum detections (0.14 ug/g range = 0.005 - 0.965) (excluding cases in which other anticoagulants were detected). Levels of bromadiolone averaged 0.31 ug/g (range = 0.03 - 1.08) when detected ($n = 28$). Data showing anticoagulant levels relative to the four raptor species with the most numerous detections are shown in Table 2.

The impact of anticoagulant exposure must extend well-beyond those cases in which acute lethal hemorrhage is the proximal cause of death. In some cases, death may follow a chain of events, or be the result of multiple factors, some of which may no longer be discernible at post-mortem examination. Sublethal hemorrhage may interfere with locomotion, predisposing animals to predation, accidental trauma, and reduced food intake. Inadequate nutrition may then predispose animals to infectious and parasitic disease, hypothermia, or poisoning with organochlorine pesticides stored in fat. Although there are potential examples of each of these scenarios in the present cases, linking these mortalities to the rodenticide exposure generally requires some speculation of varying degrees of plausibility therefore confounding meaningful objective quantification of impact. It seems clear, nevertheless, that assessment of the magnitude of anticoagulant impacts must consider these complexities.

Another possible impact to be considered is the possibility of toxic injury to the liver, the principle site of accumulation for anticoagulants. Kumar and Saxena (1993) found severe and reportedly irreversible hepatic changes that included necrosis and vacuolization of hepatocytes with enlargement and deformation of hepatocyte nuclei in rats administered a median lethal dose of bromadiolone (1.25 mg/kg). Considering the apparent high frequency of exposure in some wildlife, investigations are needed to determine histopathological effects at lesser dosage, in other species, and with other anticoagulants.

At present, anticoagulants appear to be present in the majority of great horned owls, in about half of the red-tailed hawks, and probably in substantial fractions of a number of other raptors in New York State. Similar findings were reported in a smaller survey of a variety of raptors and other non-target wildlife from California (USA) by Hosea (2000). A long-term (1983-1996) survey of anticoagulants in barn owls (*Tyto alba*) by Newton et al. (1999) in Britain recorded a frequency of detection of 26% ($n = 717$), with eight cases of anticoagulant-caused fatal hemorrhage. Additional surveys of anticoagulant exposure and mortality in raptors from other geographic regions are needed.

Table 2. Levels of anticoagulants in livers of four species of raptors in New York State 1 Jan. 1998 - 31 Dec 2001.

rodenticide ^a	\bar{x} (range) no. detections			
	<i>A. cooperii</i>	<i>B. jamaicensis</i>	<i>O. asio</i>	<i>B. virginianus</i>
brodifacoum	0.10(0.008-0.22)12	0.21(0.006-1.28)42	0.16(0.007-0.47)(8)	0.21(0.007-0.97)42
bromadiolone	0.35(0.04-0.60)5	0.23(0.08-0.50)6	0.30(0.05-0.50)3	0.23(0.05-1.08)10
diphacinone	0.10, n = 1	0.34, n = 1		
warfarin	0.10, n = 1			0.73, n = 1
chlorophacinone		0.18, n = 1		

^aIncluded in the analytical screen, but not detected were coumachlor, coumatetralyl, difenacoum, difethialone, pindone, and valone.

Both here and in our previous paper (Stone et al. 1999), we have indicated that brodifacoum appears to have the greatest potential for non-target wildlife mortality of all anticoagulants currently in use. The key to both its threat and efficacy is its unmatched physiological persistence. Remarkably, the frequent presence of brodifacoum and, to a lesser extent, bromadiolone, in some non-target wildlife species in New York has occurred despite the fact that usage of these two pesticides in the U.S. is restricted to applications in and around structures (no field uses). As other reasonably effective products less likely to intoxicate or accumulate in predators or scavengers are widely available (warfarin, chlorophacinone, cholecalciferol, bromethalin, zinc or aluminum phosphide), we suggest appropriate regulatory agencies world-wide consider further restrictions on the use of brodifacoum. We also encourage anticoagulant manufacturers to develop less persistent products.

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