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Exposure of Raptors and Waterbirds to Anticoagulant Rodenticides (Difenacoum, Bromadiolone, Coumatetralyl, Coumafen, Brodifacoum): Epidemiological Survey in Loire Atlantique (France)

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Anticoagulant rodenticides are commonly used to control rodent pests all over the world. These pesticides inhibit the vitamin K cycle in the liver, prevent blood clotting and cause death by hemorrhage. Despite the fact that they were intended for the control of commensal rodents, they may affect non target species, either directly by consumption of contaminated cereal-based baits, or indirectly by consumption of contaminated preys (secondary poisoning).

Secondary poisoning of non target species by anticoagulant rodenticides has been confirmed in many mammals and birds species, such as mustelids, foxes and birds of prey (Shore et al., 1996; Berny et al., 1997; Birks 1998; Stone et al., 1999; Stone et al., 2003; Harding 2004; Fournier-Chambrillon et al., 2004). Nevertheless, poisoning of non target species by direct consumption of anticoagulant rodenticides in the form of contaminated baits is poorly documented. Loire Atlantique is a French department well known for its wetlands and marshes in which anticoagulant rodenticides are commonly used especially against coypus (*Myocastor coypus*) because of the damage to cereal fields, rivers and water ponds banks. The aim of this study was to estimate the exposure of raptors and waterbirds to anticoagulant rodenticides in Loire Atlantique.

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Materials and Methods

Fifty-eight wild birds from Loire Atlantique (France, 44) collected by the wildlife center of the National Veterinary School of Nantes in 2003 because of distress were included in this study: 30 raptors - 4 kestrel falcons (*Falco tinnunculus*), 11 common buzzards (*Buteo buteo*), 10 barn owls (*Tyto alba*) and 5 tawny owls (*Strix aluco*) - and 28 waterbirds - 15 mallards (*Anas platyrhynchos*), 13 black coots (*Fulica atra*) and 1 common moorhen (*Gallinula chloropus*). All birds were either dead when they were brought to the center or died within one week after hospitalization. Each dead bird underwent necropsy. Cause of death was identified, if possible, and liver was sampled after gall bladder excision and kept at -18°C until analysis by the Toxicological Diagnosis Laboratory of the National Veterinary School of Nantes.

The concentrations of five anticoagulant rodenticides (brodifacoum, bromadiolone, coumafen, coumatetralyl and difenacoum) in animal liver were measured by reversed phase high-performance liquid chromatography using gradient elution and fluorimetric detection (Fauconnet et al., 1997). Anticoagulants were extracted from liver with mixtures of acetone/diethylether (90/10, v/v) and acetone/chloroform (50/50, v/v). Extracts were applied to solid-phase extraction cartridges. Method was linear within the concentration range 0.25-2.00 µg/g. Relative standard deviations of within-run and run-to-run variations were all between 5.7 and 10.3%. Limits of detection and quantitation were 0.08 and 0.25 µg/g respectively. Extraction recoveries were all above 80%.

Results and Discussion

At least one anticoagulant rodenticide was detected or quantified in the liver of 26 of the examined birds (45%). Anticoagulant rodenticides were detected or quantified in the liver of 22 raptors (73%) and 4 waterbirds (14%) (Table 1 and Fig. 1).

At least one anticoagulant rodenticide was detected in the liver (concentration ≥ 0.08 μg/g and < 0.25 μg/g) of 15 birds and quantified (concentration ≥ 0.25 μg/g) in the liver of 11 birds. Concerning the 26 contaminated birds, six of them were contaminated by two (three common buzzards, one barn owl and one kestrel falcon) or three anticoagulants (one barn owl). When an anticoagulant rodenticide was quantified in liver (concentration ≥ 0.25 μg/g), its concentration was often low (< 1 μg/g); except for one common buzzard (coumafen liver concentration = 2 μg/g) and one black coot (coumafen liver concentration = 23.52 μg/g). Among the five anticoagulant rodenticides assayed, bromadiolone was detected in the liver of 15 birds, difenacoum in the liver of 8 birds, coumafen in the liver of 5 birds, brodifacoum in the liver of 4 birds and coumatetralyl in the liver of 1 bird (Table 1).

The dependence between the exposure to anticoagulant rodenticides and the status (contaminated or uncontaminated) of the birds (raptors or waterbirds) was verified by a Chi-square test (Table 2). The ratio of raptors contaminated by at least one anticoagulant was significantly higher than the ratio of waterbirds contaminated by at least one anticoagulant ($p < 0.001$).

No necropsic signs of poisoning by anticoagulants rodenticides have been reported in dead birds. To take into account the results of all the anticoagulants without summarizing them in a synthetic variable ‘‘exposure’’, it was necessary to carry out a multivariate factor analysis (AFCM). In this analysis, each anticoagulant was considered as a factor with three modalities: undetected (< 0.08 μg/g), quantified (≥ 0.25 μg/g) and detected (≥ 0.008 and < 0.25 μg/g). In the first factorial plane, the first axis should be interpreted as the seriousness of the contamination. On the left side of this axis, the undetected modality is found; the detected and quantified modalities are found on the right side. Bird species were distributed along this axis, waterbirds in the undetected area and raptors on the quantified area. The most contaminated birds species was *Buteo buteo*, a shark bird.

Our results show that raptor exposure to anticoagulant rodenticides was high in Loire Atlantique (73% of 30 raptors). Similar findings were reported by others studies. Stone et al., (2003) detected anticoagulant rodenticides in

Table 1 Anticoagulant rodenticide concentration in liver (μg/g) according to birds species

Species	Brodifacoum	Bromadiolone	Coumafen	Coumatetralyl	Difenacoum	NCB
Common buzzard (n = 11)	ND (n = 10)	ND (n = 6)	ND (n = 7)	ND (n = 11)	ND (n = 8)	10
	D (n = 1)	D (n = 4)	D (n = 0)	D (n = 0)	D (n = 3)	
	Q (n = 0)	Q: 0.29	Q: 0.35–0.44–0.99–2	Q (n = 0)	Q (n = 0)	
Kestrel falcon (n = 4)	ND (n = 2)	ND (n = 2)	ND (n = 4)	ND (n = 4)	ND (n = 4)	3
	D (n = 1)	D (n = 2)	D (n = 0)	D (n = 0)	D (n = 0)	
	Q: 0.24	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	
Barn-owl (n = 10)	ND (n = 10)	ND (n = 6)	ND (n = 10)	ND (n = 9)	ND (n = 5)	7
	D (n = 0)	D (n = 3)	D (n = 0)	D (n = 0)	D (n = 3)	
	Q (n = 0)	Q: 0.26	Q (n = 0)	Q: 0.64	Q: 0.23–0.26	
Tawny owl (n = 5)	ND (n = 5)	ND (n = 3)	ND (n = 5)	ND (n = 5)	ND (n = 5)	2
	D (n = 0)	D (n = 2)	D (n = 0)	D (n = 0)	D (n = 0)	
	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	
Mallard (n = 15)	ND (n = 15)	ND (n = 14)	ND (n = 15)	ND (n = 15)	ND (n = 15)	1
	D (n = 0)	D (n = 1)	D (n = 0)	D (n = 0)	D (n = 0)	
	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	
Black coot (n = 13)	ND (n = 12)	ND (n = 12)	ND (n = 12)	ND (n = 13)	ND (n = 13)	3
	D (n = 1)	D (n = 1)	D (n = 0)	D (n = 0)	D (n = 0)	
	Q (n = 0)	Q (n = 0)	Q: 23.52	Q (n = 0)	Q (n = 0)	
Common moorhen (n = 1)	ND (n = 1)	ND (n = 1)	ND (n = 1)	ND (n = 1)	ND (n = 1)	0
	D (n = 0)	D (n = 0)	D (n = 0)	D (n = 0)	D (n = 0)	
	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	Q (n = 0)	
TOTAL	4	15	5	1	8	26

ND, not detected (< 0.08 μg/g); D, detected (≥ 0.08 μg/g and < 0.25 μg/g); Q, quantified (≥ 0.25 μg/g); NCB, number of contaminated birds

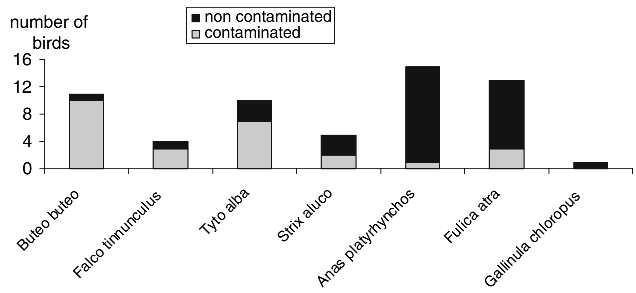

Fig. 1 Number of contaminated and uncontaminated birds according to species

Table 2 Number of contaminated and uncontaminated birds by anticoagulant rodenticides

	Contaminated birds	Uncontaminated birds	Total
Waterbirds	4	24	28
Raptors	22	8	30
TOTAL	26	32	58

49% of 265 raptors from January 1998 to June 2001 in New York. Raptors are predators or scavengers, and direct contamination after ingestion of treated baits, especially treated cereals, carrots or apples, is extremely unlikely due to their feeding diet. The raptors of this study especially eat small rodents (major species controlled by anticoagulant rodenticides) or carcasses, and secondary contamination by consumption of poisoned preys appears to be the most likely way of exposure to anticoagulant rodenticides (Merson et al., 1984). Furthermore, poisoned rodents are more vulnerable and easily captured than safe rodents because of their slower reactions increasing possible capture by predators. Exposure of other predators such as mustelids or foxes to anticoagulant rodenticides (Berny et al., 1997) are very high and well documented. Shore et al., (1996) analyzed livers of 24 polecats (*Mustela putorius*), anticoagulant rodenticides were detected in 31% of the polecats in Great Britain during 1992–1994. McDonald et al., (1998) showed that anticoagulant rodenticides were detected in 23% (9/40) of stoats (*Mustela erminea*) and 30% (3/10) of weasels (*Mustela nivalis*) in England between August 1996 and March 1997. Fournier-Chambrillon et al., (2004) estimated the exposure of 122 dead free-ranging riparian mustelids of four species (European mink – *Mustela lutreola*, Amerikan mink – *Mustela vison*, polecat – *Mustela putorius*, and European otter – *Lutra lutra*) collected between 1990 and 2002 in southwestern France: bromadiolone was found in all species and in 9% of the collected carcasses; chlorophacinone was found in two species and in 4% of the collected carcasses.

Among our samples, the common buzzard seemed to be the most contaminated species. Gastric content analysis showed that feeding diet is composed of 46% small rodents and 5% birds or mammals carcasses (Génsbøl, 1999). However, the proportion of carcasses should be greater with greater availability, which would occur especially from October to February when prey is rare. So common buzzards are more susceptible to contamination by consumption of dead preys than other diurnal raptors. Tuyttens and Stuyck (2002) reported that poisoned rodents (73%) stay above ground instead of in their burrows, increasing the risk of secondary poisoning of scavengers. To our knowledge, our study was the first one to consider the exposure of different species of raptors in France. Stone et al., (1999, 2003) showed that anticoagulant rodenticide contamination of raptors was particularly frequent in New York in great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*), two species with similar feeding diets to the common buzzard.

Anticoagulant rodenticides are used in cereal, carrot, beet or apple-based baits, which seem to be more attractive for herbivorous species than for predators. Moreover, even if the use of anticoagulant rodenticides is strictly regulated (especially by protection of baits, i.e., use in or around building, buried in holes, …), it is still possible to find some non protected bait available for non target species. In wetlands, bait must be put on fixed rafts away from banks to limit the contamination of terrestrial species but not necessarily that of waterbirds. Nevertheless our data suggested that raptor exposure was significantly greater than waterbird exposure. In relation to their diet, the major way of contamination of waterbirds was the direct consumption of treated baits (cereals, carrots or apples). Berny et al., (1997) also showed that bromadiolone was rarely detected in herbivorous species like mallards, rabbits or hare. Furthermore, the fast disappearance of bait after application limit suggests ingestion of the bait by waterbirds. However, a very high coumafen liver concentration (23.52 μg/g) was found in a black coot, probably due to direct ingestion of a relatively large amount of contaminated bait during the days prior to death.

The most frequent anticoagulant detected or quantified in the liver of birds in this study was bromadiolone. The landscape structure of Loire Altantique consists of many wetlands such as rivers, lakes, marshes and mudholes. All these wetlands are a favourable habitat for coypus, and its population is among the biggest in France. However damages caused by coypus are significant and they are considered pests. Their burrows weaken banks, and the ground collapses into the water, which gradually fill up small water ways. In addition, they cause damage to crops (maize, wheat, …), meadows, and young poplar plantations along the banks. So the fight against coypus is a permanent one in Loire Atlantique. One of the means used is the chemical fight, with bromadiolone. Even if the use of bromadiolone is regulated (regarding destruction of dead coypus and remaining treated baits), carcasses are available for scavengers, explaining the relatively large exposure of non target species to this anticoagulant rodenticide.

Our epidemiological study confirms, by inference, the exposure of non target species, such as rodent predators, to anticoagulant rodenticides. It would be interesting to determine the effects of such an exposure on the health of birds of prey. Such surveys should be encouraged in so far as they suggest secondary contamination and, in some instances, secondary poisoning to anticoagulant rodenticides. Mortality factors are particularly important to consider when they concern vulnerable species because they endanger the dynamics of populations and species viability.

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