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EVIDENCE OF SECONDARY POISONING OF FREE-RANGING RIPARIAN MUSTELIDS BY ANTICOAGULANT RODENTICIDES IN FRANCE: IMPLICATIONS FOR CONSERVATION OF EUROPEAN MINK (*MUSTELA LUTREOLA*)

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ABSTRACT: Because of the rapid decline of the endangered European mink (*Mustela lutreola*) populations in France, a national conservation program has been put into action, including research to understand the causes of decline. As part of this research, concentrations of eight anticoagulant rodenticides were examined in livers from 122 carcasses of four species of free-ranging mustelids collected between 1990 and 2002 in southwestern France. Bromadiolone residue was found in all species and 9% of the sample (one of 31 European mink, three of 47 American mink [*Mustela vison*], five of 33 polecats [*Mustela putorius*], and two of 11 European otters [*Lutra lutra*]). Liver concentrations ranged from 0.6 μg/g to 9.0 μg/g. Chlorophacinone residue was found in two species and 4% of the sample (in four of the American mink and in one of the otters), with liver concentrations ranging from 3.4 μg/g to 8.5 μg/g. Two polecats and one American mink had lesions and liver residues indicating bromadiolone was directly responsible for their death. However, most of our study animals survived secondary poisoning until they were caught; this study certainly underestimates the extent of fatal exposure of mustelids to rodenticides. Moreover, anticoagulant poisoning could increase their vulnerability to other causes of death. The current status of the endangered European mink population is such that any additional risk factor for mortality is important, and it is thus urgent to monitor and reduce the extensive use of bromadiolone and chlorophacinone against field rodents in France.

Key words: Bromadiolone, chlorophacinone, *Lutra lutra, Mustela lutreola, Mustela putorius, Mustela vison,* secondary poisoning.

INTRODUCTION

The European mink (*Mustela lutreola*) is one of 15 species of carnivores listed by the International Union for the Conservation of Nature as threatened with extinction (www.redlist.org). This species has retracted from most of its range in the last century (Youngman, 1982; Saint-Girons, 1991; Rozhnov, 1993; Maran and Henttonnen, 1995), and its distribution is still shrinking dramatically. In France, the species lost nearly half of its range during the last 20 yr and is now only present in seven départements of southwestern France (Maizeret et al., 2002). Because of this rapid decline, the French Environmental Ministry initiated a conservation program. The objectives were to stop the decline of this species in France and to initiate recovery of mink in at least a part of the area where it recently retracted. One of the main activities of this program is to implement research to understand causes of regression of the mink populations in France.

Anticoagulant rodenticides are used in

major field treatments in France during fall and winter. Bromadiolone is used extensively against coypu (*Myocastor coypus*), muskrat (*Ondathra zibethicus*), and water vole (*Arvicola terrestris*) and should only be applied by official pest control operators under strict regulatory control (www.legifrance.gouv.fr). These compounds are applied one time in places where these rodents live, that is, wetland areas, marshes, and water ponds in western France. Chlorophacinone is used against muskrat, rats (*Rattus norvegicus*), mice (*Mus musculus*), voles (*Arvicola* sp.), and other rodents and is less strictly regulated than bromadiolone. Both compounds may be used against rodents found indoors. All second-generation anticoagulant rodenticides (brodifacoum, flocoumafen, difethialone) are licensed for indoor use only (Association de Coordination Technique Agricole, 2003).

In France (1995/1996 campaign), 99% of all rodenticides sold were anticoagulants: 17% bromadiolone, 75% chlorophacinone and 7% of six other compounds (Liphatech Europe, Pont du Casse, France) distributed in 17,014 metric tons of bait. Secondary poisoning of predators by anticoagulant rodenticides via contaminated prey was recently observed in several species (Gray et al., 1994; Shore et al., 1996; Berny et al., 1997; McDonald et al., 1998; Murphy et al., 1998; Stone et al., 1999). Because of their heavy predation on rodents, mustelids may be at high risk for secondary poisoning by anticoagulant rodenticides, like several birds of prey such as barn owls (*Tyto alba*) and red kites (*Milvus milvus*) (Shore et al., 2003). Shore et al. (1996) found rodenticides in 31% of road-killed polecats (*Mustela putorius*) in western England. McDonald et al. (1998) detected rodenticides in 23% of stoats (*Mustela erminea*) and 30% of weasels (*Mustela nivalis*) in central and eastern England. Residues of brodifacoum were detected in a large number of stoats (78%), weasels (71%), and polecats (56%) after a rat- and opossum-poisoning operation in New Zealand (Murphy et al., 1998). The toxicity of rodenticides for mustelids has been demonstrated in laboratory trials (Grolleau, 1989; McDonald, 2000) and confirmed in the field (Berny et al., 1997). A 50% lethal dose of 9.2 mg/kg is reported for brodifacoum in American mink (*Mustela vison*), which is high when compared with dog (<1.0 mg/kg) and rodents (ca. 0.5 mg/kg). In order to estimate the exposure of riparian mustelids to anticoagulant rodenticides, we studied 122 dead free-ranging mustelids including European mink, American mink, polecat, and European otter (*Lutra lutra*) in southwestern France.

MATERIALS AND METHODS

Carcasses of 31 European mink, 47 American mink, 33 polecats, and 11 European otter were collected by the European Mink Network between 1990 and 2002 in eight départements of southwestern France (42°47′N to 46°22′N and 0°54′W to 4°7′W) (Fig. 1). This large network of trained trappers has been organized for the standardized study of the European mink's distribution (Maizeret et al., 2002). Members of the network were also asked to collect all dead mustelids found fortuitously in the wild.

Each animal was necropsied by trained veterinarians within 48 hr when possible or carcasses were stored frozen until necropsy. Sex was determined, the animals were weighed using an electronic letter scale (Maultronics 151 20, Maul®, Bad König, Germany), and measured (total length, head and body, foot). Age was defined as juvenile (milk teeth), subadult (adult teeth without wear and tartar), adult (teeth partly worn and with tartar), and old adult (teeth largely worn with much tartar). Physical condition was defined as very good (particularly corpulent animals with well-developed muscles and glossy coat), good (animals apparently clinically healthy), and poor (thin animals with reduced muscle tone, dull coat, and sometimes dehydrated). The quantity of fat was determined in various locations (subcutaneous, genital, mesenteric, kidney) using a scale from zero to three. A mean value was calculated and the amount of fat was defined as null (mean=[0–1.0]), poor (mean=[1.0–1.50]), moderate (mean=[1.50–2.0]), good (mean=[2.0–2.5]), and very good (mean=[2.5–3.00]). The direct cause of death was identified from gross lesions. A 1-g liver sample was extracted and frozen (−20 C) until analysis by the Toxi-

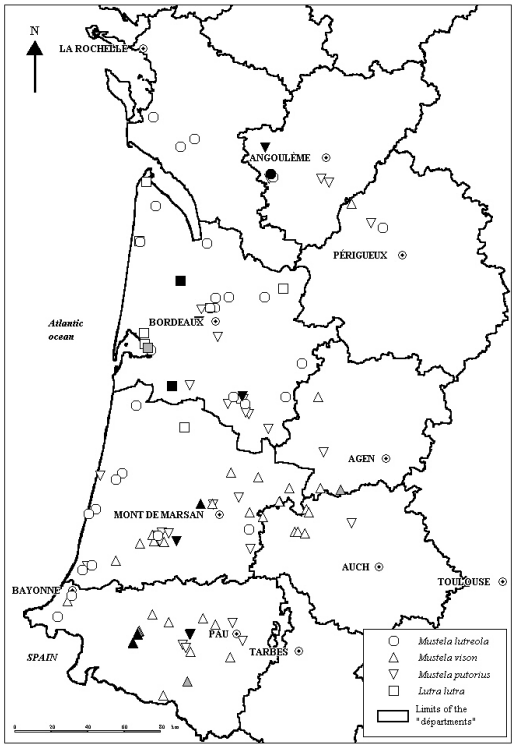

FIGURE 1. Geographic distribution of animals without anticoagulant residues (in white), with bromadiolone residues (in black), and with chlorophacinone residues (in gray) in southwestern France.

cology Laboratory at the School of Veterinary Medicine, Lyon, France.

The anticoagulant concentration in liver was determined by high-performance thin layer chromatography (Berny et al., 1995). A 1.0-g liver sample was extracted with 10 ml acetone. After centrifugation, the supernatant was separated and 1.0 ml was evaporated under a nitrogen flux. The dry residue was resuspended in 0.1 ml methanol and used for analysis. High-performance thin layer chromatography RP18 plates were used and 10-μl samples were sprayed with an Automatic thin layer chromatography sampler 3 (Camag®, Basel, Switzerland). After elution in an automatic development chamber (AMD 2, Camag®) with methanol:orthophosphoric acid (4.72 M) 9:1, a reading was made using a UV scanner II (Camag®) at 286 nm. Samples were compared to eight standard substances: brodifacoum, bromadiolone, chlorophacinone, coumachlor, coumatetralyl, difenacoum, difethialone, and warfarin. For each sample and standard, a reading was made and integrated with specific software (Wincats®, Camag). All peaks detected were integrated and further characterized by their solid-phase ultraviolet spectrum (220–390 nm). The limit of detection was 0.07 μg/g, and the limit of quantification was 0.2 μg/g. Recovery determined on spiked liver samples was 87.5% (chlorophacinone) and >90% (bromadiolone). The method was validated and appeared linear and specific under these conditions. For both compounds, the variation coefficients were <10% for recovery and linearity.

When anticoagulant was detected the animals were considered to have been exposed to anticoagulants. Anticoagulant poisoning was confirmed when liver anticoagulant concentrations were 0.2 μg/g and gross lesions (hemorrhages, unclotted blood, anemia) were observed in an animal (Berny et al., 1997). These parameters are accepted by the US Environmental Protection Agency for anticoagulant exposure and poisoning (Erickson and Urban, 2002).

RESULTS

Most animals were found dead in the wild, primarily killed by cars, except for American mink that were mostly trapped and killed for pest control (Table 1).

Bromadiolone residue was found in 11 animals (9%) of four species. Liver concentrations ranged from 0.6 μg/g to 9.0 μg/g. Chlorophacinone residue was found in four American mink and one otter (4%), and liver concentration ranged from 3.4 μg/g to 8.5 μg/g (Table 2). No other anticoagulant residue was found. Most animals exposed to anticoagulants were collected during fall and winter (Fig. 2) in five départements in the study area (Fig. 1).

All exposed animals were in good physical condition except one female polecat that was thin and dehydrated (with a fat condition defined as null).

In three animals, bromadiolone poisoning was considered the direct cause of death: one male polecat had generalized hemorrhages; the female polecat in very bad condition was found dead in a live trap and had severe anemia and dehydration; and one male American mink, trapped alive, died from massive hemorrhage. Liver concentrations of anticoagulants in these animals were 0.6 μg/g, 2.6 μg/g, and 2.0 μg/g, respectively.

For the other 13 animals with antico-

TABLE 1. Causes of death of 122 free-ranging mustelids from southwestern France.

Species	Roadkill	Trapped and killed for pest control[a]	Found dead in live traps	Killed by a carnivore	Other
European mink (*Mustela lutreola*)	13	3	3	8	4
American mink (*Mustela vison*)	3	42	1	—	1
Polecat (*Mustela putorius*)	17	7	2	3	4
European otter (*Lutra lutra*)	10	—	—	—	1

[a] European mink were killed by trappers following confusion with polecat.

agulant exposure, clinical signs and lesions were not compatible with anticoagulant poisoning. The direct causes of death were varied: the European mink was killed by a trapper following misidentification, two polecats and two otters were killed by cars, six American mink were killed for pest control, one polecat was killed by a carnivore, and one otter was drowned in a fishing net.

DISCUSSION

Our study confirms exposure of riparian mustelids to secondary poisoning by anticoagulant rodenticides in France, including rare species like European otter and endangered European mink. Exposure of European otters has not been reported previously. European mink are extremely rare today; the estimated population in France is a few hundred individuals (Maizeret, pers. comm.). Mustelids ingest anticoagulant rodenticides in contaminated prey (Murphy et al., 1998) that are not necessarily target species (McDonald et al., 1998). Because there is a delay (2–10 days) between exposure and development of clinical signs, the high proportion of exposed animals without signs is consistent with the mode of action of these products. The extensive use of bromadiolone and chlorophacinone in France provides a consistent source of contaminated prey, including target and nontarget species. A clear relationship between the amount of anticoagulant rodenticides used (i.e., area treated) and the risk of secondary poisoning was observed in France (Berny et al., 2002). Bromadiolone is primarily used in fall and late winter to reduce field rodent and coypu populations (Service Régional de Protection des Végétaux, Besançon, France). Riparian mustelids feed in aquatic habitats, the same areas where bromadiolone bait is used against coypus and muskrats, thus increasing the risk of finding contaminated prey. Moreover, poisoned rodents may leave trails of blood, stray from cover, and have slower reactions than healthy rodents, making them more vulnerable to predation (Murphy et al., 1998). Recently, it was reported that dying rodents do not remain in their burrows. Up to 73% of dead muskrats were detected above ground, therefore increasing the risk of secondary poisoning (Tuyttens and Stuyck, 2002).

We found that 13% of mustelids we studied were exposed to secondary poisoning by anticoagulant rodenticides. Three (18%) of 11 exposed animals died from bromadiolone poisoning. The animals we studied survived secondary poisoning until they were caught, before a lethal accumulation or, more likely, before significant lesions could develop because it may take up to 10 days before clinical signs and lesions are actually observed (Kolf-Clauw et al., 1995).

This study underestimated the extent of fatal poisoning of mustelids due to rodenticides. If animals do not die from second-

TABLE 2. Occurrence of anticoagulant secondary poisoning in 122 free-ranging mustelids from southwestern France.

Species	No. of animals examined	Animals with bromadiolone residue	Animals with chlorophacinone residue	Mean±SD liver concentration (μg/g)	Range of liver concentrations (μg/g)
European mink (*Mustela lutreola*)	31	1	0	5.0 (bromadiolone)	5.0 (bromadiolone)
American mink (*Mustela vison*)	47	3	4	2.7±1.3 (bromadiolone)	1.9–4.2 (bromadiolone)
				5.5±2.1 (chlorophacinone)	3.4–8.5 (chlorophacinone)
Polecat (*Mustela putorius*)	33	5	0	3.4±3.3 (bromadiolone)	0.6–9.0 (bromadiolone)
European otter (*Lutra lutra*)	11	2	1	6.6±0.8 (bromadiolone)	6.0–7.1 (bromadiolone)
				5.0 (chlorophacinone)	5.0 (chlorophacinone)

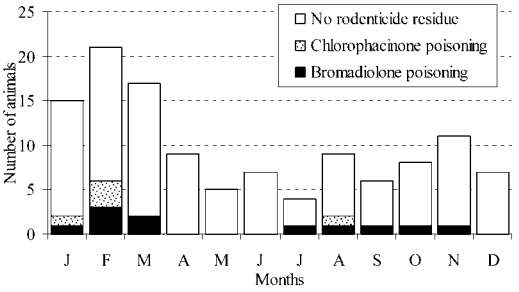

FIGURE 2. Numbers of mustelids with or without bromadiolone and chlorophacinone residues by month of examination.

ary anticoagulant poisoning following exposure, they could have increased vulnerability to other causes of death, such as vehicular collision, predation, or as observed in the affected female polecat that died in a trap. Surveys conducted on animal carcasses are biased and underestimate the true proportion of death attributable to anticoagulant poisoning (Newton et al., 1990, 1999; Shore et al., 1999; Erickson and Urban, 2002). All carcasses in our study were collected from open areas. Conversely, animals exposed to anticoagulants may be overestimated because they may be weakened and more susceptible to accidents, predation, etc. For a more accurate estimation of exposure, studies such as active monitoring on selected and well-defined populations should be conducted.

In our study, mean liver concentrations observed in several mustelids were at least 10 times higher than concentrations reported by Shore et al. (1999), who detected bromadiolone residues between 0.016 μg/g and 0.217 μg/g in road-killed polecats. However, in France bromadiolone is used against coypu over wide areas, while it is only used around buildings in other countries like Great Britain or the United States (Erickson and Urban, 2002; Shore, pers. comm.). Therefore, otters and mink in France may be exposed more often to contaminated prey than polecats in Britain and mustelids in the United States. Residue levels in liver are difficult to interpret because residues may not be closely cor-

related with mortality (Murphy et al., 1998). Grolleau et al. (1989) found liver concentrations as low as 0.23 μg/g bromadiolone in bromadiolone-poisoned ermine (*M. erminea*). In a thorough review, the US Environmental Protection Agency suggested 0.7 μg/g as a threshold for toxicity, but lower concentrations have been reported (Erickson and Urban, 2002). In our study, animals that died from anticoagulant poisoning had liver concentrations of bromadiolone higher than 0.7 μg/g. Also, liver concentrations of bromadiolone or chlorophacinone were as high as 9.0 and 8.5 μg/g, respectively, in animals without apparent lesions. This may be related to delayed onset of clinical signs and lesions. Modern rodenticides have a long biologic half-life and are bioaccumulated in secondary predators (McDonald, 2000). If exposure continues, death may occur. For instance, bromadiolone has a half-life between 170 days and 318 days. Even after a single exposure at 0.2 mg/kg in feed, liver concentrations of 0.3 μg/g were detected 200 days later (Erickson and Urban, 2002)

In our study, most cases of anticoagulant poisoning were recorded during the period of field treatments (fall and late winter), but considering the long biologic half-life of anticoagulants, exposure could be prolonged, especially for second-generation anticoagulants such as bromadiolone. As an example, Murphy et al. (1998) demonstrated persistence of brodifacoum in rats for at least 3 mo after removal of poison bait, indicating that they could provide a continuing source of exposure for mustelids long after the end of poisoning programs. In rats, chlorophacinone persists up to 30 days in the body (Kolf-Clauw et al., 1995) and bromadiolone persistence is intermediate.

Unfortunately, chlorophacinone may be used all year round because liquid concentrates can be bought and applied at any time (Berny et al., 1997). Therefore, predators could be exposed throughout the year.

We agree with McDonald (2000) that rare species are vulnerable to poisoning from anticoagulants. In the declining population of European mink, every mortality factor could push the population under the minimum viability level. It is particularly urgent to remove every direct or indirect factor of death in this species. This must be a priority for the national conservation program for this species. Therefore, field use of anticoagulant rodenticides should be reduced in France and replaced with alternative methods of pest control like trapping and use of short-acting rodenticides whenever possible. Daily collection of dead rodents in treated areas should be emphasized, as is already done in several places, and removal of unconsumed baits should also be encouraged.

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