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Authors: Berny, Philippe, and Gaillet, Jean-Roch

Source: Journal of Wildlife Diseases, 44(2) : 417-426

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-44.2.417>

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# ACUTE POISONING OF RED KITES (*MILVUS MILVUS*) IN FRANCE: DATA FROM THE SAGIR NETWORK

Philippe Berny<sup>1,4</sup> and Jean-Roch Gaillet<sup>2,3</sup>

<sup>1</sup> Toxicology Laboratory, Ecole Nationale Vétérinaire de Lyon, 1, av Bourgelat, F-69280 Marcy l'étoile, France

<sup>2</sup> Office National de la Chasse et de la Faune Sauvage, Unité de Suivi Sanitaire de la Faune, St Benoist, F-78120 Auffargis, France

<sup>3</sup> Current address: DSV de Paris 20-32, rue de Bellevue, 75019 Paris, France

<sup>4</sup> Corresponding author (email: p.berny@vet-lyon.fr)

**ABSTRACT:** Red Kites (*Milvus milvus*) are avian scavengers limited to Europe, and they currently are listed as an endangered species worldwide. Accidental poisoning is often listed as one of the threats to Red Kites throughout their range of distribution. The purpose of this article is to investigate the suspected poisoning cases reported to the French Wildlife Disease Surveillance System. Dead animals are submitted to a local veterinary laboratory for necropsy and when poisoning is suspected, samples are submitted to the Toxicology Laboratory of the College of Veterinary Medicine, Lyon, France. Over the period 1992–2002, 62 Red Kites suspected of poisoning were submitted, and poisoning was the confirmed cause of death for greater than 80% of these cases. The major toxicants found were cholinesterase inhibitors (carbamates and organophosphate insecticides) and anticoagulant compounds. The circumstances of exposure include secondary poisoning after the use of anticoagulants over vast areas to control water vole (*Arvicola terrestris*) populations, but they also include malicious poisoning with carbamates (aldicarb and cabofuran) in meat baits. Cases of poisoning vary throughout France, with observed mortality rates ranging between 0.1/100 hundred breeding pairs/10 yr and four cases/100 hundred breeding pairs/10 yr. Additional cases of poisoning likely go undetected, and our results suggest that acute poisoning is not uncommon in Red Kites and that it should be considered in the current restoration plans.

**Key words:** Acute poisoning, anticoagulant, carbamate, *Milvus milvus*, secondary poisoning.

## INTRODUCTION

The Red Kite (*Milvus milvus*) population in France has been considered stable to slightly declining (Sériot, 2005). The geographic distribution of Red Kites is limited to Europe, and the species is listed under the Red List of the International Union for the Conservation of Nature as an endangered species, Annex II of the Bern convention, and listed as a Species of European Conservation Concern (<http://milan-royal.lpo.fr>; Sériot, 2005). Red Kites are scavengers; thus, they are highly susceptible to the risk of poisoning, either by secondary exposure (e.g., ingestion of contaminated prey) or by pesticides applied on meat baits (Berny et al., 1998). Among the causes of decline, habitat modification is considered as the most important factor. Elevated mortality rates due to human impacts are also considered as threatening for this species, and the

conservation program for this species is specifically focused on identifying poisoning cases and toxicants involved (Sériot et al., 2004; Sériot, 2005).

The Toxicology Laboratory of the College of Veterinary Medicine (Lyon, France) is a member of the Wildlife Disease Surveillance System (SAGIR network) in France. Tissue samples are received daily for toxicologic analyses as part of the routine diagnostic investigations of suspected poisoning events. In the literature, many papers deal with poisoning in birds of prey, especially with insecticides (Elliot et al., 1996; Fleischli et al., 2004; Wobeser et al., 2004). The main objectives of this paper were to present data collected over 10 yr at the Toxicology Laboratory on all toxicants in Red Kites and to discuss the active ingredients involved, the circumstances of exposure, and the importance of acute poisoning in mortality rates in this unique European species.

## MATERIALS AND METHODS

The animals included in this study were collected by technicians from Office National de la Chasse et de la Faune Sauvage (ONCFS-National Hunting and Wildlife Office) and the local hunting federations. The SAGIR network is supervised by the Office National de la Chasse et de la Faune Sauvage. Hunters, technicians of the ONCFS, and the local hunting federations are responsible for the collection of wild animals found dead and their submission to the local veterinary diagnostic laboratory. As described previously (Berny et al., 1998), the network is active in all parts of France; therefore, it has a nation-wide scope.

All wild animals collected within the SAGIR network are necropsied by trained veterinarians at the local veterinary laboratory, with bacteriologic, histologic, or parasitologic examinations performed as deemed necessary. Whenever acute poisoning is suspected (e.g., sudden death of several birds, good body condition, recent use of pesticides in the area, presence of baits), biologic samples (e.g., gizzard content and liver) of the dead animals and their corresponding necropsy findings are submitted to the Toxicology Laboratory at the Veterinary College (Lyon, France). Each animal is assigned a unique identification number and accompanied by a specific case-record describing the circumstances of discovery, and any available demographic and clinical data.

At the Toxicology Laboratory, samples are analyzed according to internal procedures, published or official techniques for the analysis of residues in animal tissues, or a combination (Table 1). The Toxicology Laboratory has developed a comprehensive approach to investigate these cases (e.g., selection of animal tissues, screening techniques, clinical and circumstantial evidence of exposure; Brown et al., 2005). However, analyses are paid for by the local hunting federations; thus, funding is usually limited to investigations of wildlife poisoning incidents. Screening for poisons can include insecticides (e.g., organophosphates and carbamates, organochlorines, pyrethroids, imidacloprid, and fipronil), rodenticides, and other vertebrate poisons (e.g., anticoagulants or antivitamin K [AVKs], chloralose, crimidine, and strychnine), molluscicides (e.g., metaldehyde and methiocarb), herbicides (e.g., paraquat and diquat), avicides (e.g., chloralose), and specific drugs (e.g., euthanasia agents) as described by Brown et al. (2005). Heavy metals also may be analyzed in samples but only when clinical

evidence, exposure evidence, or both suggest the need. The laboratory has set up a quality assurance/quality control policy, and analytical investigations include the analysis of blanks, spiked samples, or both and calibration curves. Poisoning is confirmed when the exposure information (e.g., pesticide application and baiting), clinical and necropsy findings, and the presence and amount of a given poison are consistent for a poisoning event.

The information for each case submitted to the laboratory is entered into a database for future reference. The laboratory database was searched for Red Kites and Black Kites (*Milvus migrans*) suspected poisoning cases over the period 1992–2002 to retrieve information on the time of discovery, poison suspected, analytical investigations, clinical signs, lesions observed, or a combination. Results obtained in Red Kites were compared with results obtained in all wildlife species (e.g., nature of toxicant involved, circumstances of poisoning, period of exposure).

Population estimates of Red Kites were provided by the Ligue de Protection des Oiseaux (LPO-French representative of Bird Life International). Briefly, Red Kites are surveyed by visual observation and identification of nests along road transects. A specific protocol for bird watching has been set up, and it is available at <http://milan-royal.lpo.fr/>. Red Kites have been recently included in a European conservation project, and annual population estimates are available. Wintering populations are described in France, although to a limited extent (<http://milan-royal.lpo.fr/>), in most of the nesting areas, especially in the south of France or the Massif Central (center of France). In our analysis of cases, it is not possible to distinguish between wintering and nesting populations.

Anticoagulant use against water voles in the field was computed based on the data published by the regional crop protection services in the two major regions of use in France (srpv; [www.srpv-auvergne.com](http://www.srpv-auvergne.com)). Whenever possible, statistical tests were conducted. For case distributions (e.g., monthly repartition and proportion of various toxicants), chi-square tests were used, with a level of significance  $P < 0.05$ .

## RESULTS

Over the study period, 4,323 suspected poisoning cases of wildlife were investigated at the toxicology laboratory. Among them, Eurasian Buzzards (*Buteo buteo*)

TABLE 1. Analytical techniques used for toxicology screening at the College of Veterinary Medicine Toxicology Laboratory (and published references).

Group	Compound	Detection <sup>a</sup>	Confirmation	Reference <sup>b</sup>	LOD (µg/g or mg/l) <sup>c</sup>
Anticoagulant	8	HPTLC-UV	HPLC-UV/ Fluo	Berny et al. (1995, 2006)	0.05 (L) 0.01 (Pl)
Carbamate/	5 compounds	HPTLC-UV	HPLC-UV	Berny et al. (1999)	1 (GC, L)
Organophosphate	15 compounds	HPTLC-UV	GC-MS	AFSSA	0.001 (GC, L)
Organochlorine	12 compounds	GC-ECD	GC-MS	AFSSA	0.0001 (GC, L)
Pyrethroid	5	GC-ECD	GC-MS	AFSSA	0.001 (GC, L)
Fipronil		HPLC-UV	GC-MS	Jennings et al. (2002)	0.01
Convulsant	Strychnine	HPTLC-UV	GC-MS	Braserton and Johnson (2003)	0.1 (GC)
	Crimidine				
Molluscicide	Metaldehyde	Colorimetric	GC-MS		1 (GC)
Herbicide	Paraquat	UV	HPLC-UV	Blake et al. (2002)	0.1 (GC, K)
Euthanasia	Pentobarbital	GC-MS		Hooijerink et al. (1998)	0.1 (Pl)
	T61®				
Avicide	α-Chloralose	Colorimetric	GC-ECD	Brown (1996, 2005)	1 (GC)
Heavy metal	Pb, Cd, Cu	AAS		AFSSA	0.001 (L, K)
	Hg	Cold vapor			0.005 (Pl)
	As	AAS		AFSSA	(L, K) (GC, L, K)

<sup>a</sup> AAS = atomic absorption spectrometry; ECD = electron capture detection; Fluo = fluorescence detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HPTLC = high-performance-thin layer chromatography; MS = mass spectrometry; UV = ultraviolet.

<sup>b</sup> AFSSA = official method developed by the French Agency for Food Safety.

<sup>c</sup> LOD = limit of detection; L = liver; Pl = plasma; Bl = blood; GC = gizzard content; K = kidney.

were the most common birds of prey received (314 cases). Red Kites (62 cases) were the second major bird of prey, followed by Black Kites (40 cases).

There is no statistical difference between monthly distribution of Red Kite cases and other wildlife cases (chi-square test,  $P=0.21$ ,  $df=23$ ). Most cases were submitted during autumn and spring, with a limited number of cases submitted during summer (Fig. 1). The proportion of confirmed poisoning cases was significantly higher in Red Kites (82%) compared with other wildlife species (54%) (binomial test,  $P<0.01$ ). The number of confirmed Red Kite poisoning cases for groups of pesticides is given in Figure 2; most cases were associated with AVK and insecticide poisoning. Among AVKs (27 confirmed cases), bromadiolone (0.2–5.6 µg/g wet weight) was detected in 24 cases and chlorophacinone (0.9–5.2 µg/g wet weight) in three cases. Among cholinesterase inhibitors (21 confirmed cases),

carbofuran was detected in 14 cases, mevinphos in five cases, and aldicarb in two cases. The other products detected were α-chloralose (four cases), imidacloprid (one case), lindane (one case), and lead (one case). There was no evidence of poisoning with herbicides, drugs for euthanasia, or any other pesticide.

Geographic distribution of poisoning cases and of Red Kites is depicted in Figure 3. There were a few individual cases reported from neighboring areas outside of the known distribution of Red Kite populations (Fig. 3b). Mortality rates based on population data (Fig. 3b) and identified poisoning cases vary between 0.1/100 hundred breeding pairs/10 yr to four cases/100 hundred breeding pairs/10 yr. No statistical comparison between areas could be conducted due to the limited number of cases. There is evidence, from both maps, that AVKs are widely used in some areas (Fig. 3a), with some differences: scattered use in central

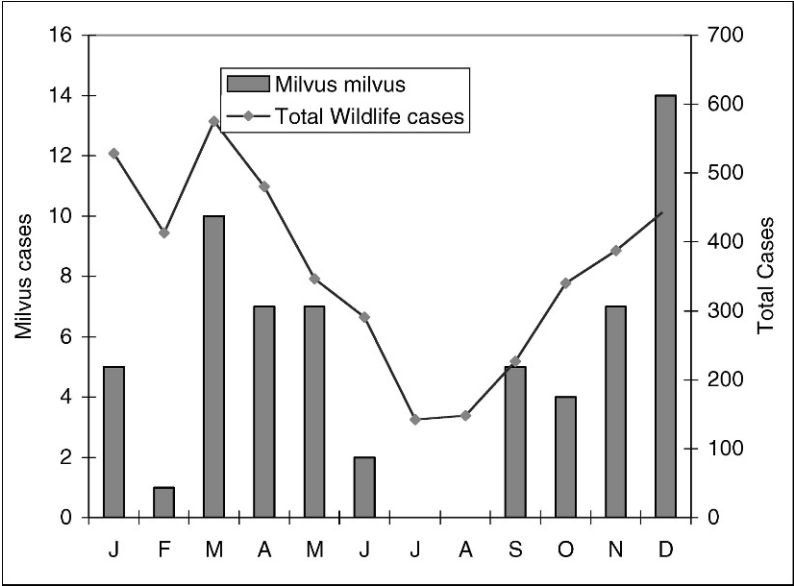


FIGURE 1. Monthly distribution of suspected poisoning cases in Red Kites (*Milvus milvus*) and in wildlife (less red kites) in general between 1992 and 2002 in France.

France and continuous use in eastern France. Most AVK poisoning cases occur in the eastern France (Fig. 3b), although the local population of Red Kites is lower.

DISCUSSION

The number of wildlife cases submitted to the Toxicology Laboratory is substantial

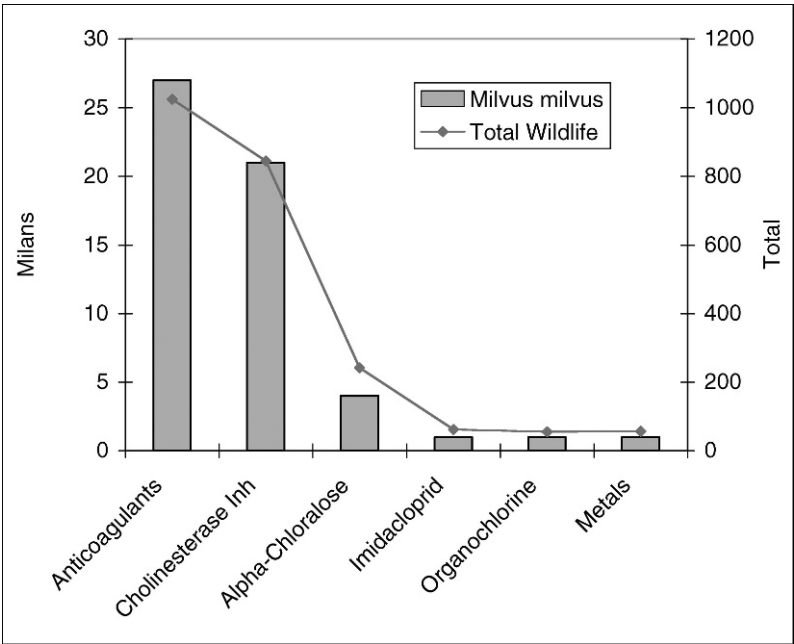
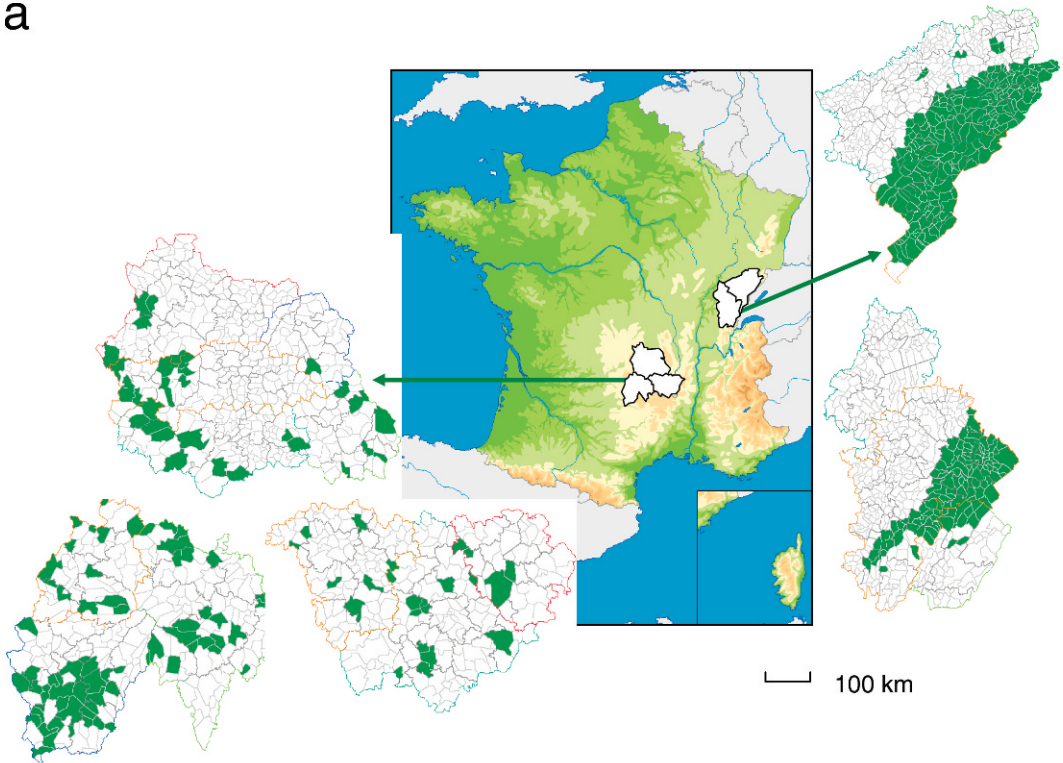


FIGURE 2. Toxic compounds detected in Red Kites (*Milvus milvus*,  $n=62$ ) and in wildlife (less Red Kites,  $n=4,326$ ) in general (number of cases) between 1992 and 2002 in France ( $n$  = number of cases submitted).

a



b

**Population :**

- >200 pairs
- 50-200 pairs
- 10-50 pairs
- 1-10 pairs

**Poisoning :**

- > 10 cases
- 2-10 cases
- 1 case

Green : Anticoagulant  
Brown : other cases

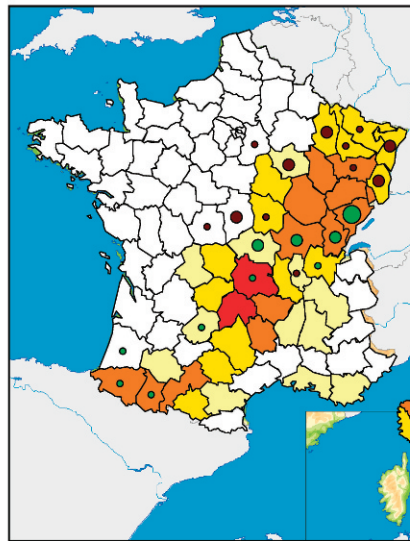


FIGURE 3. Geographical distribution of (a) major areas (green areas) treated with bromadiolone against field voles (*Arvicola terrestris*) and (b) Red Kite (*Milvus milvus*) population and poisoning cases between 1992 and 2002 in France. Population data were obtained from Ligue de Protection des Oiseaux (French section of Birdlife International) ([www.milan-royal.lpo.fr](http://www.milan-royal.lpo.fr)).



(400–450/yr), and it provides a reasonable database on poisoning cases in wild mammals and birds. As stated previously (Berny et al., 1998), the French network relies primarily on hunters for case submission, and analyses are paid for by hunting associations; thus, most species submitted for investigation are game species. As a comparison, in the Wildlife Incident Investigation Scheme (WIIS) reports in the United Kingdom, birds of prey are very commonly sent to the lab for investigation (WIIS, 2003; [www.defra.gov.uk](http://www.defra.gov.uk)), with an average of 20–22 Red Kites/yr (versus six in France); there are approximately 370–380 breeding pairs in Great Britain, which is approximately 10 times less than reported in France in 2000 (Sériot, 2005; <http://milan-royal.lpo.fr/>). In the UK, the system does not rely on private funding; thus, there is an obvious bias in case collection in France. Nevertheless, the case-series described in this article provides some valuable data on an endangered species for which there are only a few published toxicologic investigations. The majority of cases occur during early spring and autumn; therefore, wintering kites may be exposed as well. It is possible that in early spring, snow cover limits food in the areas where Red Kites are found. Considering the importance of AVK poisoning, the monthly distribution of poisoning cases is also driven by the use of bromadiolone against *Arvicola terrestris* at that time of the year, especially in autumn and early spring (Berny et al., 1997).

Investigation of suspected poisoning cases in wildlife may be complex and time-consuming. The strategy developed by the WIIS in the UK, for example, is based on necropsy, with a wide multi-residue chemical analysis (Brown et al., 1996, 2005). Although this approach may yield many interesting results, it is expensive and time-consuming. In the French system, necropsy is performed locally to be conducted as soon as possible after collection, and appropriate samples may

be kept frozen and submitted to the toxicology laboratory for chemical investigations. Clinical (when possible) and necropsy findings may suggest a specific pesticide poisoning. As stated by Fairbrother (1996), acute exposure to carbamate/organophosphate pesticides is usually suggested by the discovery of dead animals in good condition (e.g., adequate body mass and fat), with signs of convulsions (e.g., tightened claws), pulmonary edema, and diarrhea. Because of the delay between body collection, necropsy and submission to the toxicology laboratory, investigation of acetylcholinesterase inhibition is no longer considered, although it is commonly used in other case series (Elliott et al., 1996; Wobeser et al., 2004). Spontaneous reversal, as commonly described with carbamates, and lack of inhibition in the brain in case of sudden death (Wobeser et al., 2004) are also pitfalls of the cholinesterase inhibition method, resulting in a high proportion of false negative cases: these authors found four negative cases out of seven with confirmed exposure (carbournan identified in the gizzard). For example, Guitart et al. (1999) describe a case of death with aldicarb, in which the bait was found in the esophagus only, confirming the high toxicity and rapid onset of signs. Therefore, identification of suspected poisoning cases relies on chromatographic determination of carbamates and organophosphates as described above, with cholinesterase inhibition on a thin layer chromatography plate to confirm the activity of the compound identified (Berny et al., 1999).

Poisoning by AVKs is also easily suggested by severe hemorrhagic lesions and lack of clotting. Because the network is only dealing with spontaneous submission of dead animals, the number of poisoning cases should not be compared with prevalence or incidence of poisoning. The high proportion of confirmed poisoning among the suspected cases indicates that the selection process is relevant and

effective; toxicants not detected in the cases submitted to the Toxicology Laboratory represent less than 20% of the submitted cases. To have a reasonable idea of the importance of poisoning among the various causes of death, systematic investigations should be conducted in bird populations, as reported by Wendell et al. (2002) for raptors admitted at the Colorado State Veterinary Teaching Hospital. Wendell et al. (2002) identified toxicosis as the primary cause of death in about 2% of the birds, which is fairly consistent with data published by the SAGIR network regarding game species (Lamarque et al., 1999). In White-tailed Sea Eagles (*Haliaeetus alvicilla*) in Greenland, one case of lead poisoning was identified among 12 reported deaths (Krone et al., 2004). Considering the limited number of birds included in this series, the proportion of poisoning cases (8%) may not be significant.

Among the toxicants found in Red Kites in this study, cholinesterase inhibitors (21 cases) and anticoagulant rodenticides (27 cases) were predominant, findings that corresponded to a previous survey in birds of prey (Berny et al., 1998). Both toxicant groups are reported as major toxicants in birds of prey, although seldom in the same study. For example, Fleischli et al. (2004) reported investigations of 355 anticholinesterase poisoning cases from more than 35,000 wildlife deaths in the United States, with fanfur and carbofuran as primary toxicants. In New York, Stone et al. (2003) reported only on anticoagulant poisoning in raptors. In the UK, WIIS (2003) reports indicate that insecticides are commonly detected in raptors, with carbofuran, mevinphos, and aldicarb as the most common compounds. In France, we found a similar pattern for toxicants, which may reflect the European homologations of pesticides. These insecticides are highly toxic to birds of prey (toxic doses <10 mg/kg), and they are available as concentrated formulations. Indeed, they are available as micropellets for soil

treatment at 5% or 10% active ingredient, or as a liquid concentrate (mevinphos) (ACTA, 2005). Despite strict regulations on the sale and use of such pesticides, they can be easily purchased. There is evidence that most cases reported in this article are illicit poisoning cases (Berny et al., 1998), as often reported elsewhere in the world (Elliott et al., 1996; Wobeser et al., 2004), and even in Red Kites in Spain (Sergio et al., 2005). Reports from European surveys on pesticide incidents in wildlife also describe deliberate abuse as the major cause of pesticide poisoning in most species (de Snoo et al., 1999; Van Oers et al., 2005). Birds of prey, especially scavengers, suffer from a bad reputation, and they are often incorrectly associated with negative events in game species populations. As described by Villafuerte et al. (1998), major population decreases due to infectious diseases (in hares in their study) may be falsely attributed to predation by Red Kites; this may result in illicit hunting and poisoning of birds of prey. Because these raptors are scavengers, they are easily poisoned by meat baits containing insecticides, and because carbamates and organophosphates may be highly persistent in the environment, poisoning with baits may be possible over several days (Wilson et al., 2002). As suggested by Sergio et al. (2005), predators and scavengers are often poisoned by hunters because they are considered to be competitors. Population management strategies should also include, as they suggest, information and implication of inhabitants and hunters to understand the benefits of scavengers.

Anticoagulant poisoning is also commonly described in birds of prey, as a result of secondary poisoning, but mostly in nocturnal birds such as owls (Newton et al., 1990, 1999). This is especially true for second-generation products, such as brodifacoum or bromadiolone (Eason et al., 2002; Erickson and Urban, 2002). Usually, animals poisoned by AVKs die several days after exposure; thus, studies relying on



animal carcasses may be biased and underestimate the true proportion of death due to AVKs poisoning (Newton et al., 1990, 1999; Erickson and Urban, 2002; Shore et al., 2003). In France, bromadiolone is widely used against field voles (*Arvicola terrestris*), coypu (*Myocastor coypu*) and musk rat (*Ondathra zibethicus*). There is evidence that the use of bromadiolone is associated with exposure or poisoning of predators and scavengers in the same environment (Berny et al., 1997; Fournier-Chambrillon et al., 2004). Shore et al. (2003) also showed that many mustelids found dead had detectable residues of anticoagulants, although death could be attributed to another cause.

In this study, we observed an association between AVK poisoning and its use. For example, bromadiolone is applied on many scattered areas in the center of France, but over a vast, contiguous area in eastern France. As a result, bromadiolone poisoning of Red Kites in eastern France is much more pronounced. It has also been shown recently (Giraudoux et al., 2006) that field voles store wheat bait and that they may consume it several days or weeks after use; hence the proportion of voles caught with high residues of bromadiolone is almost 100% for over 10 days after field application, thereby exposing Red Kites for prolonged periods. These data indicate that there is some concern about the widespread use of AVKs and their potential side effects on nontarget species. For an endangered species, this is even more of a concern. Our data suggest that if AVKs have to be used, they should be applied on small dispersed areas to avoid contamination of an entire population of voles and consequently, repeated poisoning of scavengers feeding on poisoned rodents.

The other toxicants we measured account for only a few percent of the poisoning cases. Only  $\alpha$ -chlordane resulted in several poisoning cases, but much less than reported in the UK (WIS, 2003).  $\alpha$ -Chlordane is commonly used in France

as a bird control product (ACTA, 2005). Its toxicity is important in many bird species, but it is not labeled for use against birds of prey. Because it is only sold as a cereal-based bait, exposure of birds of prey should not occur often (ACTA, 2005). There is no evidence that this compound accumulates in the food chain (Brown et al., 1996). Secondary poisoning may occur when scavengers eat rodents or birds killed by chlordane poisoning, by direct ingestion of the gizzard content. It is our hypothesis that illicit meat baits were also prepared with this product and ingested by Red Kites. There was also one case of acute exposure to imidacloprid. This is, to our knowledge, the first report of exposure of a bird of prey to this insecticide. We do not have any information on the toxicity of imidacloprid in birds of prey, and, in other bird species, it may vary greatly with acute oral lethal dose 50 from 14.5 mg/kg to 152 mg/kg ([www.inra.fr/agritox](http://www.inra.fr/agritox)).

In the cases investigated, there was no heavy metal poisoning case reported, although heavy metals are commonly suggested as potentially toxic to Red Kites, especially due to the consumption of bullet fragments in prey (Mateo et al., 2003). As stated herein, the policy is to investigate the cause of death and not to determine whether the birds were exposed to a given toxicant. Based on necropsy findings, lead poisoning, for example, is not often suspected, and heavy metal residues are not determined systematically because of limited funding.

In conclusion, it seems that poisoning is a common feature in Red Kites, with two major groups of compounds involved: cholinesterase inhibitors and anticoagulant rodenticides. It is difficult to determine the impact of poisoning on the population. Based on our data, mortality due to acute poisoning may reach a few individuals per thousand, but we suspect this number is underestimated. Acute poisoning remains a potential threat on a weakened population, especially when AVKs are used on large areas or as a

result of illicit poisoning. It would certainly be extremely valuable to develop a specific monitoring system for this endangered species, because it is already undergoing a restoration program in France.

#### ACKNOWLEDGMENTS

We thank J. Sinardet and D. Vey for excellent technical assistance in the laboratory; ONCFS and Hunting federations, who provided funding for the analyses reported here; and Ligue de Protection des Oiseaux (French representative of Birdlife International) (Mionnet), who provided the data on Red Kite population status over the study period.

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*Received for publication 31 July 2006.*