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Exposure of predatory and scavenging birds to anticoagulant rodenticides in France: Exploration of data from French surveillance programs

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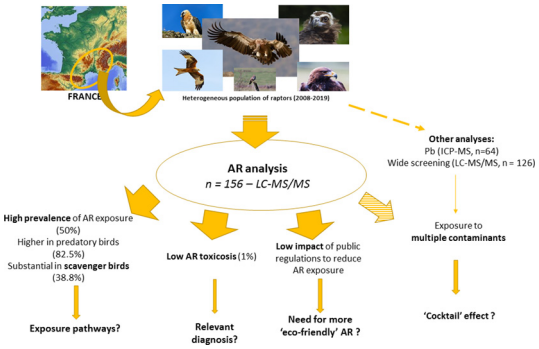
HIGHLIGHTS

- Persistent & bioaccumulative anticoagulant rodenticides (AR) are a threat to raptors.
- AR exposure prevalence was 82.5% in predatory birds and 38.8% in scavenger birds.
- Exposure to multiple ARs was common as were differences in exposure among species.
- Public policy mitigation measures did not reduce second-generation AR exposure.
- Diagnosis of AR toxicosis is complex and the 100 ng/g ww threshold was problematic.

GRAPHICAL ABSTRACT



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ABSTRACT

Wild raptors are widely used to assess exposure to different environmental contaminants, including anticoagulant rodenticides (ARs). ARs are used on a global scale for rodent control, and act by disruption of the vitamin K cycle that results in haemorrhage usually accompanied by death within days. Some ARs are highly persistent and bioaccumulative, which can cause significant exposure of non-target species. We characterized AR exposure in a heterogeneous sample of dead raptors collected over 12 years (2008–2019) in south-eastern France. Residue analysis of 156 liver samples through LC-MS/MS revealed that 50% (78/156) were positive for ARs, with 13.5% (21/156) having summed second-generation AR (SGAR) concentrations >100 ng/g ww. While SGARs were commonly detected (97.4% of positive samples), first-generation ARs were rarely found (7.7% of positive samples). ARs were more frequently detected and at greater concentration in predators (prevalence: 82.5%) than in scavengers (38.8%). Exposure to multiple ARs was common (64.1% of positive samples). While chlorophacinone exposure decreased over time, an increasing exposure trend was observed for the SGAR brodifacoum, suggesting that public policies may not be efficient at mitigating risk of exposure for non-target species. Haemorrhage was observed in 88 birds, but AR toxicosis was suspected in only 2 of these individuals, and no difference in frequency of haemorrhage was apparent in birds displaying summed SGAR levels above or below 100 ng/g ww. As for other contaminants, 17.2% of liver samples (11/64) exhibited Pb levels compatible with sub-clinical poisoning

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(>6 μg/g dw), with 6.3% (4/64) above the threshold for severe/lethal poisoning (>30 μg/g dw). Nine individuals with Pb levels >6 μg/g dw also had AR residues, demonstrating exposure to multiple contaminants. Broad toxicological screening for other contaminants was positive for 18 of 126 individuals, with carbofuran and mevinphos exposure being the suspected cause of death of 17 birds. Our findings demonstrate lower but still substantial AR exposure of scavenging birds compared to predatory birds, and also illustrate the complexity of diagnosing AR toxicosis through forensic investigations.

1. Introduction

Wild raptors are widely used in Europe for biomonitoring trends in environmental contamination, natural resource management and as sentinels for human health, although harmonization of pan-European monitoring programs has yet to be achieved (Badry et al., 2020; Espín et al., 2016; Gómez-Ramírez et al., 2014). In free-ranging raptors, many studies have assessed exposure to various environmental contaminants, including lead (Pb) (Descalzo et al., 2021; Finkelstein et al., 2012; Krone, 2018; Mateo, 2009; Monclús et al., 2020), veterinary drugs (e.g. diclofenac) (Bean and Rattner, 2018; Cuthbert et al., 2011, 2014; Gómez-Ramírez et al., 2020; Swan et al., 2006), pesticides (e.g., organophosphorus and carbamates) (Allen et al., 1996; Botha et al., 2015), and anticoagulant rodenticides (ARs) (Albert et al., 2010; Murray, 2011, 2017, 2020; Rattner and Harvey, 2021; Thomas et al., 2011). Anticoagulant rodenticides have been widely used since the 1950's for pest control (Wardrop and Keeling, 2008). Widespread resistance of rodents to some first generation ARs (FGARs; e.g., warfarin) became apparent in the 1950's, which eventually resulted in the development of second-generation compounds (e.g., bromadiolone, brodifacoum, difenacoum, difethialone and flocoumafen; Buckle et al., 1994; Goulois et al., 2017; Lefebvre et al., 2016; Marquez et al., 2019). ARs disrupt the vitamin K cycle by inhibiting vitamin K epoxide reductase (VKOR), leading to impaired blood coagulation that can be fatal to both target pest species and non-target species (reviewed in Rached et al., 2020).

Second-generation ARs (SGARs) are persistent, bioaccumulative and toxic compounds, and are currently the most commonly used products to control rodent populations. Non-target exposure to ARs may be primary (e.g., direct consumption of baits) or secondary (e.g., consumption of intoxicated or dead rodents that had been exposed to ARs), and can include transfer through food webs (Hindmarch and Elliott, 2018). Multiple AR residues reflect repeated exposure that could lead to adverse effects (Rattner and Harvey, 2021) and also reflect the complexity of exposure pathways (Hindmarch and Elliott, 2018; Rattner et al., 2014). Contamination of non-target raptors with SGARs is globally widespread (Albert et al., 2010; Murray, 2011, 2017, 2020; Rattner and Harvey, 2021; Thomas et al., 2011; Van den Brink et al., 2018). Prevalence of ARs exposure in wild predatory birds and mammals was recently estimated as 58%, reviewing data from North America (Canada, USA), Europe (UK, Denmark, France and Spain) and New Zealand between 1984 and 2015 (López-Perea and Mateo, 2018). Data are more scarce for scavenging birds.

In France, ARs are authorized as biocides to be used by professionals and the public in urban and suburban settings (European Biocidal Product Regulation N°°528/2012) and are also used as plant protection products (PPP, e.g. for the control of the European water vole *Arvicola amphibius* in crops and meadows up to 2020) (Giraudoux et al., 2012). Chlorophacinone was used as a PPP in France till the end of 2010 (Ministère de l'Agriculture et de la Pêche, 2007), and bromadiolone was authorized for this use till its recent withdrawal (21 February 2020 - see https://www.anses.fr/fr/system/files/phyto /decisions/SUPERCAIDA_PREX_2015-1399_D.pdf) by the French Agency for Food, Environmental and Occupational Health & Safety. Other ARs are only authorized as biocides. Regulatory measures were also implemented in an attempt to reduce SGAR exposure of non-target fauna through biocidal use. Starting in 2013 within the European Union, rodenticide baits to be placed indoors or in and around buildings must be in tamper-resistant bait stations. These stations limit primary exposure of non-target species by physically preventing access to baits (Berny et al., 2014; Nakagawa et al., 2015; Suárez and Cueto, 2018), but regrettably still may permit bait access of non-target species of a similar size as pest rodents. To our knowledge, the success of these public policies has not been thoroughly assessed.

Diagnosis of AR lethal intoxication is complex, generally associating post-mortem lesions suggestive of AR poisoning (i.e., haemorrhages) and quantifiable residues in the liver (Murray, 2018). However, there is no consensus on the toxicity threshold to distinguish between sublethal exposure and poisoning (Rattner and Harvey, 2021). An often cited toxicity threshold for SGAR levels of >100 to 200 ng/g liver wet weight (ww) has been suggested to be potentially lethal (Newton et al., 1999), although both field observations and modeling efforts indicate that SGAR toxicity and lethality can occur at much lower levels (Thomas et al., 2011), and species-specific differences in sensitivity are notable (Rattner and Harvey, 2021). It was also recently suggested that due to large differences in molecular weight and VKOR inhibitory potency, summing of ARs should be limited to second-generation ARs, and it may be appropriate to present exposure data on both a summed mass basis and summed molar basis (Rattner et al., 2020; Rattner and Harvey, 2021).

Herein we present findings characterizing prevalence and factors affecting AR exposure in raptors sampled in France, through analysis of residues in liver of dead predatory and scavenging birds collected over a 12-year period by several bird of prey surveillance programs. The effects of public policies to mitigate risk to non-target fauna were also assessed. We examined diagnosis of AR intoxication using both hepatic AR concentration and molar values that may better represent exposure. As these avian samples were part of broad biomonitoring programs, results of other toxicological analyses (lead, some pesticides and pharmaceuticals) are briefly mentioned to emphasize concomitant exposure to multiple contaminants in wild birds.

2. Material and methods

2.1. Collection of postmortem data: use of a French surveillance database

Raptors (in most instances carcasses) were collected over a 12 year period (2008–2019) from several bird of prey surveillance programs [e.g., LIFE GYPCONNECT that include partnerships with associations such as the Bird Protection League (LPO), and the National Centre for Veterinary Toxicological Information (CNITV); Supplemental Table S1]. Sampling was principally focused on mountainous areas of southeastern France, from the eastern Pyrenees to Alps, in the regions of Auvergne-Rhône-Alpes, Occitanie and Provence-Alpes-Côte d'Azur, especially in the regional natural parks of Baronnies, Grands Causses, Vercors and Verdon, and southern department of Aude, which are the working areas for LIFE GYPCONNECT (see Fig. 1 for the summary of origins of all individuals). Scavenging birds accounted for the majority of samples (see Table 1 for the number of individuals per species collected).

Carcass collections were performed by field agents, usually on the day of the animal death or within a few days thereafter. Carcasses were frozen (−18 °C), and subsequently thawed and necropsied within a variable time frame (several days to a month) by veterinarians of

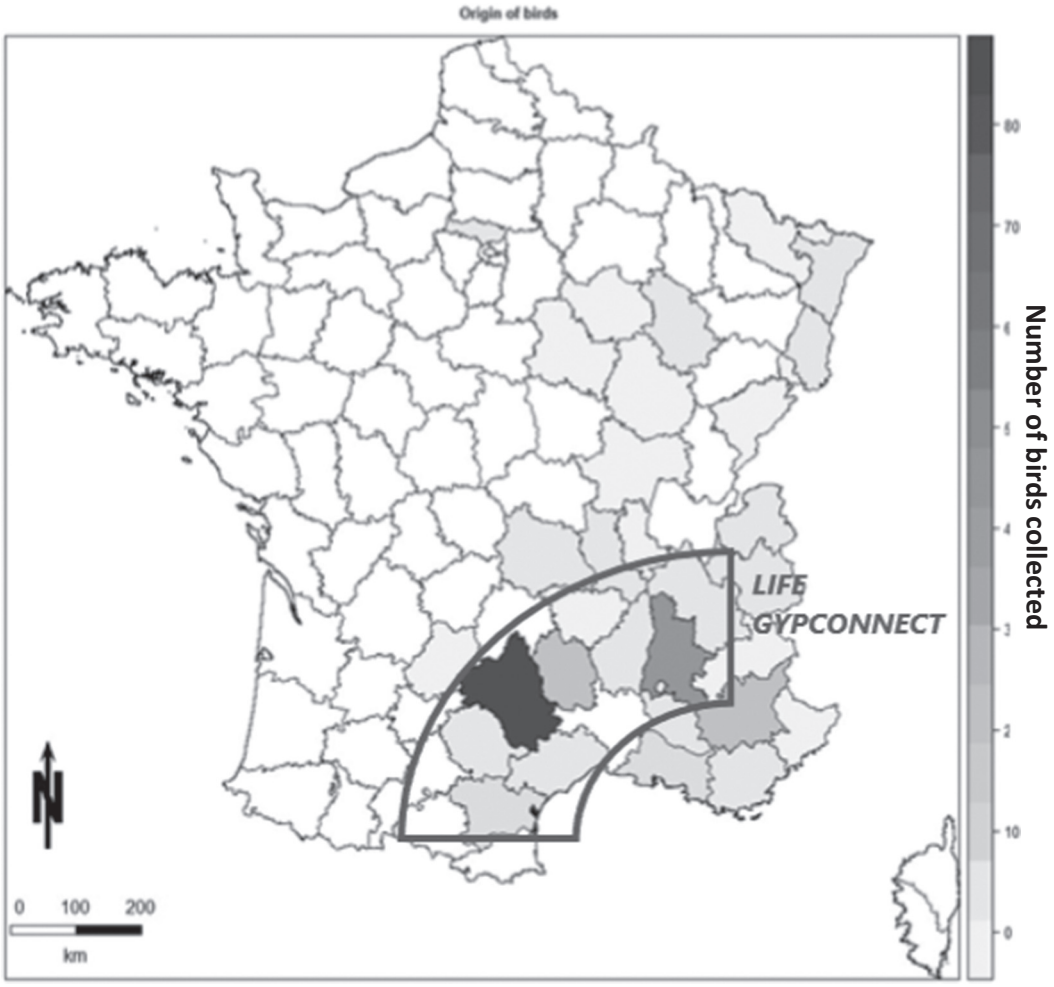

Fig. 1. Number of birds collected per department ($n = 245$) (*gray frame: working area of LIFE GYPCONNECT*).

CNITV. While those veterinarians were not pathologists, necropsies were supervised by a senior veterinarian with over 20 years of experience in bird of prey necropsy. For each specimen, history of the carcass (collection location, date of discovery, description of the area) was provided, and information on the species, sex, as well as descriptions of the external aspects and of all organs were compiled during necropsies. Radiographs were performed, and gastric content, liver, kidney, muscle and bone were sampled. Only liver samples were used for this study.

Findings were coded in a database, with details on lesions, results of toxicological analysis and possible cause of death. All necropsy reports in the database were analysed to identify elements for further exploration. Parameters which were not systematically documented (e.g. age of birds and state of decomposition) were not coded for further analysis. Presence/absence of haemorrhages was coded based on keywords in the report ("haemorrhage", "bleeding", "anaemia", "bruising", "haematoma","blood"), and reports with at least one of these keywords were read to determine whether or not the animal had displayed haemorrhages. Conclusions of reports were analysed, with cause of death classified in six categories [e.g., Electrocution, Trauma, Intoxication, Other (external cause; suspected drowning, poisoning), Other (internal cause; suspicion of a tumoral process, cardiopathy, infectious disease, etc.), and Unknown].

2.2. Sample analysis

AR residue analysis of liver samples was conducted using a previously described liquid chromatography with tandem mass spectrometry (LC-MS/MS) method (Fourel et al., 2017a). Analytes quantified included 3 first-generation ARs (i.e., warfarin, coumatetralyl, chlorophacinone) and 5 s-generation ARs (SGARs) used in Europe (i.e., bromadiolone, brodifacoum, difenacoum, flocoumafen and difethialone). Limit of quantification (LOQ) was 2 ng/g ww. All analyses (samples collected between 2008 and 2019, stored at −80 °C) were performed between 2016 and 2020 using the same analytical procedures.

As collected birds were part of broad toxicological surveillance programs, it should be noted that some of the samples were also sent to other external laboratories for lead (Pb) analysis (French departmental laboratory of Drôme, LDA 26) by inductively coupled plasma mass spectrometry (ICP-MS) and for a broad toxicological screening with a panel of more than 500 environmental contaminants (mostly pesticides and pharmaceuticals, laboratory Ecoloxie-La Voulte) using LC-MS/MS. Those labs are accredited by a French committee (COFRAC). Results from those other analyses will only be briefly mentioned in this paper.

2.3. Statistical analyses

Descriptive statistics and graphs were generated with R software (version 3.5.1) associated with RStudio (version 1.1.453). Concentrations of ARs in liver were expressed as ng/g ww, and also converted to pmol/g ww (or equivalent nmol/kg ww) using the molecular weight for each AR (PubChem: chlorophacinone = 374.8 g/mol bromadiolone = 527.4 g/mol, brodifacoum = 523.4 g/mol, difenacoum = 444.5 g/mol, difethialone = 539.5 g/mol). As warfarin, coumatetralyl and flocoumafen concentrations were consistently below the LOQ, those ARs were excluded from statistical analyses.

Table 1
Species, sample size, foraging behaviour and frequency of exposure to ARs of raptors collected between 2008 and 2019[a].

Feeding habit + A3:K42	Species	n	AR exposed	CHLORO	BROMA	BFC	DFC	DFT	Multiple ARs
			(%)	N exposed					
				(% of tested samples N) (% of AR exposed)					
Scavengers	Griffon vulture (*Gyps fulvus*)	90	29/90 (32)	3 (3) (10)	17 (19) (59)	10 (11) (34)	10 (11) (34)	13 (14) (45)	13 (14) (45)
	Cinereous vulture (*Aegypius monachus*)	17	13/17 (76)	2 (12) (15)	10 (59) (77)	5 (29) (38)	6 (35) (46)	6 (35) (46)	7 (41) (54)
	Bearded vulture (*Gypaetus barbatus*)	9	3/9 (33)	0	2 (22) (67)	1 (11) (33)	2 (22) (67)	2 (22) (67)	2 (22) (66)
	Bonelli's eagle (*Aquila fasciata*)	7	5/7 (71)	0	4 (57) (80)	4 (57) (80)	5 (71) (100)	4 (57) (80)	4 (57) (80)
	Red kite (*Milvus milvus*)	16	16/16 (100)	0	14 (87) (87)	15 (94) (94)	9 (56) (56)	7 (44) (44)	14 (87) (87)
	Golden eagle (*Aquila chrysateo*)	7	7/7 (100)	0	6 (86) (86)	7 (100) (100)	6 (86) (86)	7 (100) (100)	7 (100) (100)
Predators	Common buzzard (*Buteo buteo*)	3	1/3	0	1	1	1	1	1
	Black kite (*Milvus migrans*)	1	1/1	0	0	1	0	0	0
	Eurasian eagle-owl (*Bubo bubo*)	2	2/2	1	2	2	2	2	2
	Hen harrier (*Circus cyaneus*)	2	0/2	0	0	0	0	0	0
	Eurasian hobby (*Falco subbuteo*)	1	0/1	0	0	0	0	0	0
	Short-toed snake eagle (*Circaetus gallicus*)	1	1/1	0	1	0	0	0	0
Total		156	78/156 50	6 (4) (8)	57 (37) (73)	46 (29) (59)	41 (26) (53)	42 (27) (54)	50 (32) (64)

Abbreviations: chlorophacinone, CHLORO; bromadiolone, BROMA; brodifacoum, BFC; difenacoum, DFC; difethialone, DFT; not analysed, NA.
[a] % were reported when sample size was greater than 5.

For descriptive results of the levels of ARs (analytes with <50% of samples below the LOQ), the Kaplan-Meier method was used to estimate the extremes of the mean (Helsel, 2005). Lower bound was calculated replacing values below the LOQ by 0.02 ng/g ww, while upper bound was obtained by replacing those values by the LOQ (i.e., 2 ng/g ww). For each species, the range of values, number of animals above LOQ and number of animals with a sum of SGARs above the literature threshold of 100 ng/g ww (Newton et al., 1999) were described.

To assess AR exposure by feeding regimen, a simple classification was implemented. All vultures were categorized as scavengers, while other raptors, including facultative scavengers (e.g., Golden eagle, *Aquila chrysaetos*), were classified as predators. Chi-squared test was used to detect differences in AR exposure between scavengers and predators for prevalence data (e.g., AR detection rate) and *t*-test for continuously distributed variables (e.g. summed SGARs concentration). Other factors, such as presence of multiple ARs, species (retaining only species with sample sizes ≥5 individuals), gender and area of discovery (department), were also tested. Parametric tests (t-test) were used for sample size >30 individuals, while non-parametric procedures (Wilcoxon or Kruskal-Wallis, depending on the number of categories) were used for smaller sample size comparison of AR concentration.

To evaluate the effect of change in public policies in France related to AR usage on detection frequency, samples were grouped into 3 different key temporal periods: 2008–2010 (end of chlorophacinone use as a plant protective product in 2010), 2011–2013 (new regulations on SGARs in 2013, brodifacoum, difenacoum and difethialone only to be used in bait boxes starting from 2013) and 2014–2019. Differences in exposure among those 3 key periods were assessed for each AR using Fisher's exact test.

The relation between levels of SGARs found in the liver samples and evidence of AR intoxication (i.e., presence/absence of haemorrhage, for each individual) was examined using non-parametric Wilcoxon test. As trauma and electrocution were considered as possible biases for the presence of haemorrhages, individuals with such mortality classifications were removed for additional analysis. To test the commonly reported threshold of 100 ng/g ww, birds positive for SGARs were then classified into 2 groups, namely those with summed hepatic SGARs <100 ng/g ww or ≥ 100 ng /g ww, and compared using Chi-squared test (all individuals) or Fisher's Exact test (birds with "Unknown" cause of death), based on the presence/absence of haemorrhages. As trauma is a common cause for haemorrhage, relationship between AR exposure and presence of haemorrhage in birds that died from trauma was also tested through Fisher's Exact test.

For liver lead (Pb) concentrations, toxicity thresholds reported in literature were used to classify the results [subclinical poisoning (2 to <6 μg/g ww, corresponding to 6 to <18 μg/g dry weight), clinical poisoning (6–10 μg/g ww, i.e., 18–30 μg/g dw), and severe poisoning compatible with death (>10 μg/g ww or > 30 μg/g dw)] (Descalzo et al., 2021; Pain et al., 2019).

3. Results

3.1. Study population, causes of death

In total, 245 birds were necropsied, with the most represented species being Griffon vulture (*Gyps fulvus*; $n = 139$, 56.7% of samples), Red kite (*Milvus milvus*; $n = 32$, 13.1%), Cinereous vulture (*Aegypius monachus*; $n = 26$, 10.6%) and Bearded vultures (*Gypaetus barbatus*; $n = 14$, 5.7%). Scavengers accounted for the majority of collected samples (73.1%). Excluding cases for which the cause of death could not be determined (i.e., unknown, $n = 62$, 25.3%), the principal causes of mortality were trauma and electrocution ($n = 71$, 29% and $n = 50$, 20.4%, respectively). Two birds (one with trauma, one electrocuted) were found alive but were euthanised due to poor prognosis for survival. Other reported causes were internal disorders (mainly infectious processes, 13.1%), intoxication (lead or other contaminants, i.e., carbofuran, mevinphos or pentobarbital, 9.4%) and other external causes (drowning, envenomation, 2.9%).

3.2. AR exposure

Liver samples were not available for all specimens recorded in the database (absent due to consumption of part of the carcass before collection or advanced decomposition), and thus only 156 samples were analysed for ARs (63.7% of individuals).

3.2.1. Prevalence and concentrations of ARs

Of the 156 liver samples analysed, 78 (50.0%) were found positive for at least one AR at a concentration exceeding the LOQ (Table 1). While the FGAR chlorophacinone was present in only 6 birds (4% of all samples, 7.7% of positive samples), SGARs were found in nearly all positive samples (76/78, 97.4%), with bromadiolone being detected most frequently ($n = 57$, 73.1% of positive samples).

Concentrations of individual compounds ranged up to 231.4 ng/g ww (442.1 pmol/g ww; brodifacoum, Red kite) (Table 2). Brodifacoum had the highest median concentration, followed in descending order by difenacoum, bromadiolone and difethialone (Fig. 2). However, concentrations for these 4 SGARs were not different (Kruskal-Wallis $X^2 = 5.40$, df = 3, $p = 0.1450$). Chlorophacinone levels were lower when compared to each of these SGARs (Kruskal-Wallis rank sum test, $p < 0.0001$ followed by pairwise Wilcoxon test with Bonferonni correction: chlorophacinone vs brodifacoum or bromadiolone, or difenacoum or difethialone, $p < 0.0001$ for each comparison).

Only 21 individuals (13.5% of samples) had summed SGAR concentrations in liver exceeding 100 ng/g ww (see Table 2 for the details of prevalence per species). When this 100 ng/g ww threshold of summed SGARs for all samples was examined on a molar basis (i.e., using brodifacoum as a reference, 100 ng brodifacoum/g liver ww = 191 pmol/g liver ww), these same 21 individuals had summed SGAR molar values exceeding 191 pmol/g ww. Only 7 birds had a concentration of an individual SGAR above the commonly used threshold of 100 ng/g ww (i.e., 9% of the positive samples).

3.2.2. Factors affecting AR exposure

3.2.2.1. Foraging behaviour. Anticoagulant rodenticide residues were more frequently detected in predatory birds (33 of 40 samples, 82.5%) than in scavenging birds (45 of 116 samples, 38.8%) (Chi-squared test, $X^2 = 21.01$, df = 1, $p < 0.001$). The summed concentration of SGARs was also greater in predators than in scavengers (*t*-test, $t = 5.45$, df = 41, $p < 0.0001$, 95% confidence interval (CI) for difference: 52.9–115.2 ng/g ww), averaging 94.57 ng/g ww in predators vs 10.48 ng/g ww in scavengers (Fig. 3B). Nevertheless, while concentrations of SGARs were low for the majority of scavengers, levels of summed SGARs in 16 of 116 samples (13.8%) were outliers (values above Quartile 3 + 1.5× interquartile range), with 12 (10.3%) being extreme points (Quartile 3+ 3× interquartile range). For these 12 individuals with extreme values (6 Griffon vultures, 4 Cinereous vultures and 2 Bearded vultures), summed SGAR concentrations in liver ranged from 30.2 to 169.6 ng/g ww (57.7–338.1 pmol/g ww), with 4 individuals exceeding 100 ng/g ww (Supplemental Table S2).

3.2.2.2. Number of ARs detected. For liver samples with quantifiable AR residues, 64.1% ($n = 50$ of 78) contained two or more compounds. Multiple AR residues were more frequently present in predatory birds (28 of 33, 82.5%) than in scavenging birds (22 of 45, 48.9%) (Chi-squared test, $X^2 = 9.19$, df = 1, $p = 0.0024$). Summed SGAR concentrations were higher in birds with multiple ARs (Wilcoxon test, W = 91.5, $p < 0.0001$), and among the 21 birds (including 4 scavenger birds) with summed SGARs concentration above 100 ng/g ww, all had multiple SGAR residues, with 16 of them exposed to the four SGARs, and 2 of these 16 samples also containing chlorophacinone residues.

3.2.2.3. Species. The concentration of summed SGARs differed among species with sample sizes ≥5 individuals (Kruskal-Wallis rank sum test, $X^2 = 63.45$, df = 5, $p < 0.001$). Levels were greater in Golden eagles and Red kites compared to Bearded, Cinereous and Griffon vultures, respectively (pairwise Wilcoxon test with Bonferonni correction: Golden eagle vs vulture species $p = 0.0241$, 0.0223 and < 0.001, and for Red kite vs vulture species $p = 0.0100$, 0.0440 and < 0.001) (Fig. 3A), further supporting the aforementioned difference in exposure between predatory and scavenger birds.

3.2.2.4. Other factors. Concentrations of summed SGARs did not differ between sexes (*t*-test, $t = -1.014$, df = 102.96, $p = 0.3132$). Birds were categorized by the geographic region (i.e., department) where they were found, but there was no difference among departments (sample size ≥5 individuals) in summed SGAR concentrations for either scavenging (5 departments, Kruskal-Wallis $X^2 = 4.0515$, df = 4, $p = 0.3991$) or predatory birds (2 departments, Wilcoxon test, W = 32, $p = 0.3690$).

3.3. Temporal trends of AR exposure

A decreasing trend in the proportion of individuals exposed to chlorophacinone was observed (Fisher's exact test, $p = 0.0041$, df = 2) when birds were grouped according to three key periods of changing patterns of AR use ($n = 3$ of 17 for 2008–2010, $n = 2$ of 24 for 2011–2013, and $n = 1$ of 115 for 2014–2019), with a significant difference between 2008 and 2010 and 2014–2019 (pairwise comparison of proportions using Fisher's test with Bonferroni correction, $p = 0.0213$, df = 1, Odds Ratio = 0.043 [0.0008–0.5747]) (Fig. 4). In contrast, an increasing trend for brodifacoum was observed among these 3 key periods (Fisher's exact test, $p = 0.0398$, df = 2; but no difference for pairwise comparisons). For the other ARs, hepatic residue concentrations were similar among these 3 periods (Fisher's exact test for bromadiolone, $p = 0.5389$; for difenacoum, $p = 0.378$; for difethialone, $p = 0.2743$; for summed SGAR, $p = 0.2609$).

3.4. Evidence of AR intoxication

Of 156 birds tested for ARs, only 130 were examined for the presence of haemorrhage at necropsy because of carcass decomposition of the 26 other specimens. Eighty-eight (88) of those 130 birds displayed signs of haemorrhage (67.7%). None were initially diagnosed with AR toxicosis, but for 9 birds, cause of death was undetermined. Six (6) of those 9 birds were positive for ARs: 2 had low levels of summed SGARs (2.1 and 2.2 ng/g ww) and were not further considered, but the remaining 4 birds (all Red kites) had summed SGAR levels ranging from 27.3 to 191.9 ng/g ww. Levels of ARs and the clinical picture for those 4 individuals are described in Table 3 and Fig. 5, respectively. While the clinical picture does not favour coagulopathy in birds 1 and 2, lesions reported in birds 3 (Fig. 5) and 4, combined with high levels of summed SGARs, are suggestive of AR toxicosis in those birds (2/156, i.e., 1.3% of tested birds actually died of AR toxicosis).

There was no difference in summed SGAR concentrations between birds with or without haemorrhage (non-parametric Wilcoxon rank Test, W = 1681, $p = 0.3814$). For the remaining subset of birds excluding those with trauma and electrocution, summed SGAR concentrations did not differ between those with or without apparent haemorrhage for all birds (Wilcoxon rank Test, W = 365.5, $p = 0.2584$) and for the subset of species with ≥5 individuals (i.e., Griffon vulture, Cinereous vulture and Red kite - Wilcoxon rank test, W = 103, 6 and 10, respectively, and *p*-values = 0.4523, 1 and 1, respectively). When examining data for only those birds with cause of death classified as "Unknown" ($n = 20$; avoiding bias), summed SGAR concentrations did not differ between those with or without apparent haemorrhage (Wilcoxon test, W = 49, $p = 1$).

No difference was found between summed SGARs >100 ng/g and presence of haemorrhages, for samples involving all individuals (Chi-squared test, $X^2 = 0.057$, df = 1, p-value = 0.8117) or when excluding

Table 2
Hepatic residues of anticoagulant rodenticides (in ng/g ww or *pmol/g ww*) in raptors collected in France between 2008 and 2019.

Feeding habit	Species	N	CHLORO	BROMA	BFC	DFC	DFT	∑SGARs
			Kaplan-Meier mean (in ng/g ww)					
			Kaplan-Meier mean (in pmol/g ww)					
			Range (ng and pmol/g ww)					
			Detects > LOQ					
			–					>100 ng.g-1/detects
Scavengers	Griffon vulture *(Gyps fulvus)*	90	0.4–2.3 *0.9–6.0* LOQ-16.3/*43.5* 3/90	2.92–4.50 *5.5–8.5* LOQ-86.0/*163.1* 17/90	2.6–4.3 *4.9–8.2* LOQ- 63.5/*121.3* 10/90	0.7–2.4 *1.5–5.5* LOQ-11.1/*25.0* 10/90	0.7–2.4 *13.1–44.5* LOQ-10.9/*20.2* 13/90	6.9–13.6 *13.3–26.67* LOQ-128.0/*246.2* 29/90 2/29
	Cinereous vulture *(Aegypius monachus)*	17	0.7–2.5 *2.0–6.6* LOQ-9.1/*24.3* 2/17	11.94–12.64 *22.6–24.0* LOQ-61.0/*115.7* 10/17	5.0–6.2 *9.5–11.9* LOQ-39.3/*75.1* 5/17	5.4–6.7 *12.2–15.1* LOQ-54.6/*122.8* 6/17	8.4–9.7 *15.6–18.0* LOQ-71.4/*132.3* 6/17	30.8–35.3 *59.9–69.0* LOQ-169.6/*338.12* 13/17 2/13
	Bearded vulture *(Gypaetus barbatus)*	9	/ / LOQ 0/9	2.4–3.9 *4.5–7.4* LOQ-11.9/*22.56* 2/9	2.2–4.0 *4.3–7.6* LOQ-19.9/*38.0* 1/9	1.7–3.2 *3.8–7.3* LOQ-11.2/*25.2* 2/9	3.8–5.4 *7.1–10.0* LOQ-27.7/*51.3* 2/9	10.1–16.5 *19.7–32.3* LOQ-42.2/*81.4* 3/9 0/3
Predators	Red kite *(Milvus milvus)*	16	/ / LOQ 0/16	29.1–29.4 *55.2–55.7* LOQ-121.9/*231.1* 14/16	37.5–37.6 *71.6–71.9* LOQ-231.4/*442.1* 15/16	20.2–21.0 *45.5–47.1* LOQ-95.3/*214.4* 9/16	20.0–21.0 *37.1–38.9* LOQ-122.3/*226.7* 7/16	106.8–108.9 *209.4–213.6* 2.4/*4.2*–365.3/*705.4* 16/16 7/16
	Bonelli's eagle *(Aquila fasciata)*	7	/ / LOQ 0/7	14.1–15.0 *26.8–28.40* LOQ-35.4/*67.1* 4/7	12.8–13.4 *24.5–25.6* LOQ-71.0/*135.6* 4/7	17.1–17.7 *38.5–39.8* LOQ-88.0/*198.0* 5/7	10.9–11.7 *20.1–21.7* LOQ-35.2/*65.2* 4/7	54.9–57.7 *109.9–115.4* LOQ-224.0/*455.5* 5/7 1/5
	Golden eagle *(Aquila chrysateo)*	7	/ / LOQ 0/7	32.7–32.9 *61.9–62.5* LOQ-112.0/*212.4* 6/7	53.1–53.1 *101.4–101.4* 3.1/*5.9*–98.0/*187.2* 7/7	24.2–24.5 *54.5–55.1* LOQ-63.0/*141.7* 6/7	19.5–19.5 *36.1–36.1* 5.3/*9.8*–33.0/*61.2* 7/7	129.4–130.0 *253.9–255.1* 21.3/*36.3*–204.4/*410.0* 7/7 6/7
	Common buzzard *(Buteo buteo)*	3	/ / LOQ 0/3	2.6–3.9 *4.9–7.4* LOQ-7.7/*14.6* 1/3	20.8–22.1 *39.7–42.2* LOQ-62.3/*119.0* 1/3	5.9–7.2 *13.2–16.2* LOQ-17.6/*39.6* 1/3	49.8–51.1 *92.3–94. 8* LOQ-149.4/*276.9* 1/3	79.1–84.3 *150.2–160.6* LOQ-237.0/*450.1* 1/3 1/1
	Hen harrier *(Circus cyaneus)*	2	/ / LOQ 0/2	/ / LOQ 0/2	/ / LOQ 0/2	/ / LOQ 0/2	/ / LOQ 0/2	/ / LOQ 0/2 0/0
	Eurasian eagle-owl *(Bubo bubo)*	2	1.8–2.8 *4.7–7.3* LOQ-3.5/*9.3* 1/2	88.4–88.4 *167.6–167.6* 71.6/*135.8*–105.2/*199.5* 2/2	44.4–44.4 *84.7–84.7* 13.9/*26.6*–74.8/*142.9* 2/2	46.5–46.5 *104.5–104.5* 30.7/*69.1*–62.2/*139.9* 2/2	73.8–73.8 *136.8–136.8* 30.2/*56.0*–117.4/*217.6* 2/2	253.0–253.0 *493.6–493.6* 180.0/*351.1*–326.0/*636.2* 2/2 2/2
	Black kite *(Milvus migrans)*	1	/ / LOQ 0/1	/ / LOQ 0/1	/ / 3.0/*5.7* 1/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / 3.0/*5.7* 1/1 0/1
	Short-toed snake eagle *(Circaetus gallicus)*	1	/ / LOQ 0/1	44.0 *83.4* 44.0/*83.4* 1/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / 44.0/*83.4* 1/1 0/1
	Eurasian hobby *(Falco subbuteo)*	1	/ / LOQ 0/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / LOQ 0/1	/ / LOQ 0/1 0/0

Abbreviations: chlorophacinone, CHLORO; bromadiolone, BROMA; brodifacoum, BFC; difenacoum, DFC; difethialone, DFT; limit of quantification, LOQ.

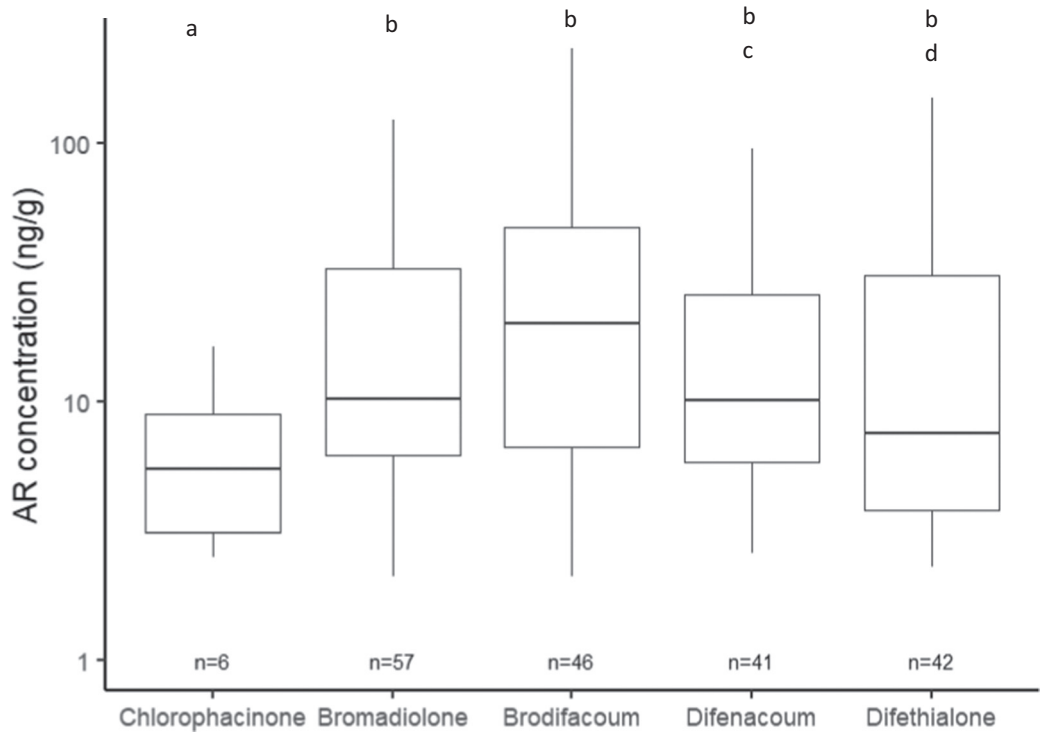

Fig. 2. Distribution (boxplot: minimum, first quartile, median, third quartile, and maximum) of detected quantities of each AR in birds; compounds not sharing common letters are different ($p < 0.001$).

bias and keeping only birds with "Unknown" cause of death ($n = 13$; Fisher's Exact test, OR = 0.527, $p = 1$).

Thirty-nine (39) of the 130 birds examined for the presence of haemorrhages died from trauma, and 35/39 displayed signs of haemorrhage (i.e., 89.7% of birds with trauma). AR exposure was detected in 15/35 birds, with 7 birds having summed SGARs concentrations ≥100 ng/g ww. No relationship was apparent between AR exposure and presence of haemorrhage in birds that died from trauma (Fisher's Exact test, OR = 0.756, p = 1).

3.5. Other contaminants

Lead concentration was determined in 64 liver samples representing 8 species. Evidence of elevated Pb exposure (>6 μg/g dw) was apparent in 11 individuals (17.2%) and levels exceeding the threshold of severe/lethal poisoning (>30 μg/g dw) occurred in 4 individuals (6.3%). In the necropsy reports, lead intoxication was described as the suspected cause of death for these 4 individuals. Of the liver samples with elevated Pb exposure (>6 μg/g dw, $n = 11/64$), 9 (81.2%) were also positive for ARs. All ARs detected were SGARs, with summed concentrations ranging from 2.5 to 224 ng/g ww. Seven (7) individuals had multiple SGARs, and 3 of them displayed summed SGAR levels >100 ng/g. Pb intoxication was suspected in 3 of those birds with multiple SGAR exposure, and the 4 others died from electrocution ($n = 3$) or from trauma (n = 1).

Broad toxicological screening was performed on 126 birds, of which 18 exhibited positive results. For those birds, it was concluded that cause of death was consistent with pesticide or pharmaceutical intoxication (18/126, 14.3%). Carbofuran was detected in 13 samples (13/18, i.e., 72.2% of intoxicated birds – 10.3% of screened birds) and mevinphos in 4 samples (4/18, 22.2% of intoxicated birds – 3.2% of screened birds). One bird was positive for pentobarbital that had not been euthanised, potentially from consumption of tissue from euthanized livestock that had been mistakenly dropped off at a feeding station. Of these 18 positive samples, 12 had been analysed for AR residues and 9 (75.0%) of these were positive (SGARs present in 7, 58.3%). Summed SGAR concentrations ranged from 3.0 to 213.5 ng/g ww. Multiple SGARs were found in 5 samples.

4. Discussion

4.1. Causes of death of the study population

Scavengers, and especially Griffon vultures, were the most frequently represented samples. This is likely due to a sampling bias, as vultures are widely monitored in France, and Griffon vultures were the first to be reintroduced starting in the 1980s through several programs and National Action Plans (Ministère de l'environnement, de l'énergie et de la mer, 2017) resulting in a large population ($n = 1544$ reproductive pairs in 2014, with census in the Pyrenees counting 1254 reproductive pairs in 2019). Another likely source of bias was apparent when comparing numbers of samples collected over the years (more samples from 2016 to 2019) as the LIFE GYPCONNECT program was most active doing carcass collection, necropsy and toxicological analyses during that period.

The principal causes of death reported for all birds were trauma (28.6% of all samples) and electrocution (20%). Trauma was often related to the collision of the bird with a wind turbine, vehicle or power line. Collision with wind turbines is a well-known threat to wild birds (Barrios and Rodríguez, 2004; Hunt et al., 2017), and was reported to account for up to 41% of casualties in areas directly surrounding wind farms. Collision with power lines results either in trauma or electrocution, and represents another major threat for wild birds (Barrientos et al., 2012; Jenkins et al., 2010).

4.2. Characterizing exposure to ARs in wild birds

4.2.1. Prevalence of AR exposure in raptors from France

All available liver samples collected from dead raptors were systematically analysed, without any prejudgment of potential AR exposure. This systematic monitoring may allow less bias in the estimation of the prevalence of AR exposure than incident-based surveillance that is

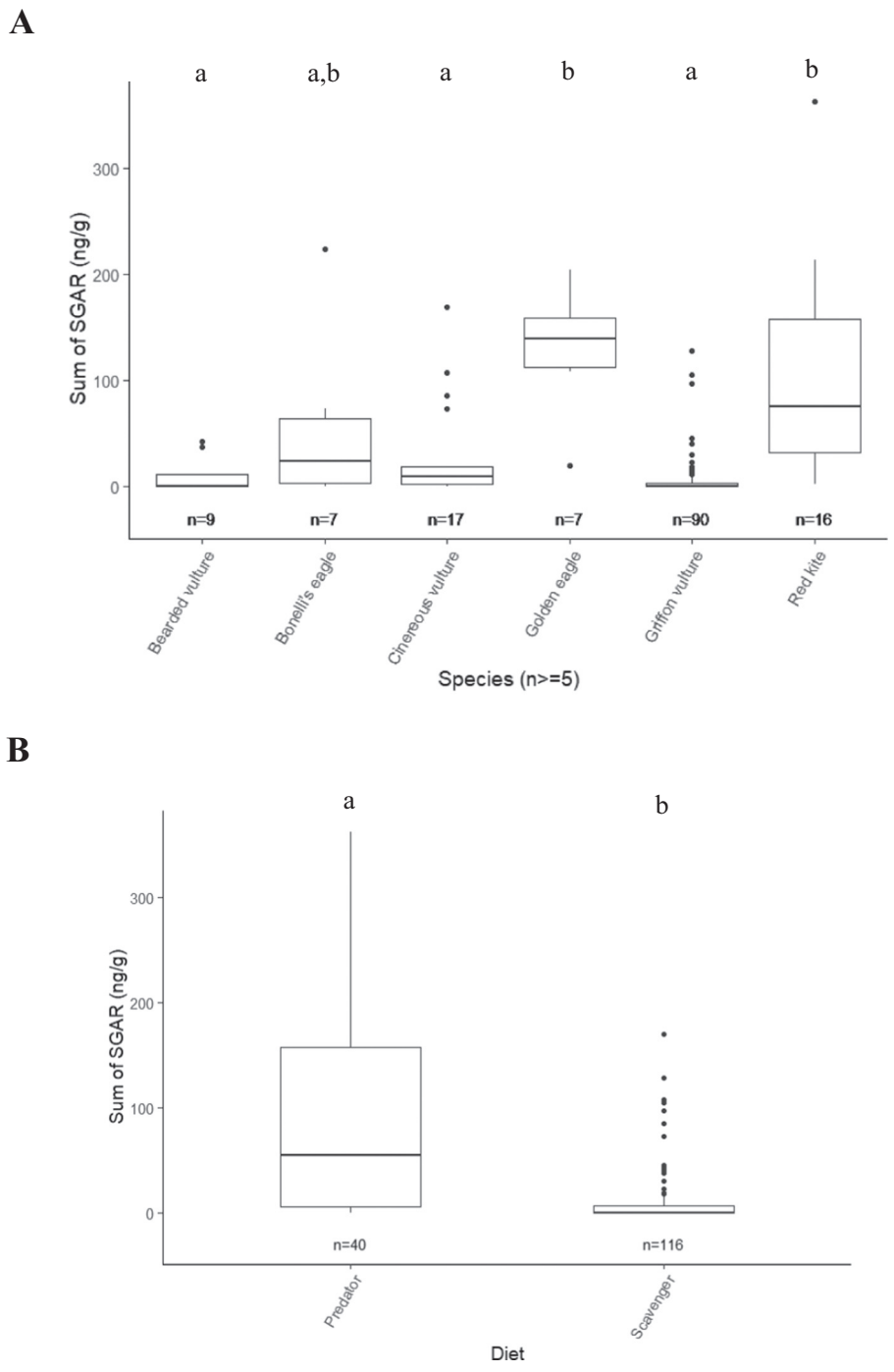

Fig. 3. A/ Summed SGAR concentrations per bird species (boxplot) and B/ summed SGAR concentration by foraging behaviour (boxplot); individual SGARs<LOQ were assigned a value of zero. Species or foraging behaviour groups not sharing a common lower case are different ($p < 0.05$). SGAR, second-generation anticoagulant rodenticide; LOQ, limit of quantification.

sometimes performed and certainly overestimates this prevalence (Millot et al., 2017; Murray, 2011, 2017, 2020; Sánchez-Barbudo et al., 2012). Yet, half of the samples (78/156) were positive for ARs. This is consistent with the mean exposure rate calculated in the review from López-Perea and Mateo (2018), who estimated a prevalence of ARs in raptors of 58% (López-Perea and Mateo, 2018). More recent data from the UK indicates a higher prevalence of 66.8% in common Kestrels (Roos et al., 2021). However, both those rates are based on results obtained from predatory birds. In our study, prevalence of AR exposure in predatory birds (82.5%) was higher than previously reported rates, but was calculated based on a small sample size ($n = 40$). Prevalence in scavenger birds (38.8% - $n = 116$) was lower, but still quite substantial. To our knowledge, this is the first report of prevalence of AR exposure in scavenging birds calculated on a large sample size. Future work focusing on the prevalence of AR exposure in scavenging birds could add clarity this observation.

Detected ARs were mainly SGARs, which is consistent with their greater environmental persistence when compared to FGARs (Buckle et al., 1994; Giraudoux et al., 2006). Warfarin and flocoumafen were not detected in any of the samples, and they were not sold in France during the study period (https://simmbad.fr/public/servlet/accueilGrandPublic.html). While coumatetralyl is authorized for use in France, it was not detected.

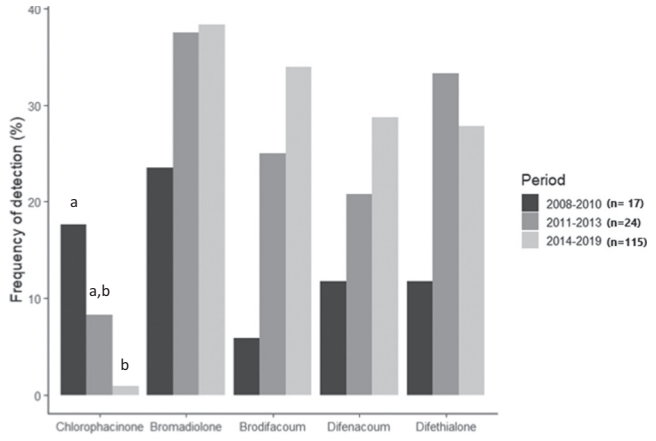

Fig. 4. Detection frequency of each AR per time period; chlorophacinone detection frequency differed ($p < 0.05$) between 2008 and 2010 and 2014–2019 as indicated by different letters superscripts.

Levels of SGARS were mostly low, with only 13.5% of samples above the commonly used threshold of 100 ng/g ww. This suggests a relatively prevalent exposure of wild birds in the zone of the study (i.e. southeastern France), but mainly at low levels.

4.2.2. Factors affecting AR exposure

4.2.2.1. Exposure of predatory vs scavenging birds: different pathways?. As expected, the proportion of AR-positive samples was greater in predators than scavengers. Predatory birds are likely to be directly exposed to ARs through consumption of intoxicated rodents, which may be weakened and motionless, lose their nocturnal disposition and positive thigmotactic behaviour, and are therefore easy prey (Cox and Smith, 1992; Frankova et al., 2017; Littin et al., 2000). Exposure of predatory birds could also occur through the consumption of AR-contaminated non-target mammals, reptiles, and birds (e.g., passerines, waterbirds) (Hindmarch and Elliott, 2018; Nakayama et al., 2019; Rattner et al., 2014). It should however be noted that one limit of this study was that the opportunistic nature of some predator species (e.g., Golden eagle is a facultative scavenger obtaining much of its diet through predation) was not taken into account, as well as some species-specific feeding habits (e.g., Short-toed snake eagle whose diet is mainly reptiles, not rodents).

Even if prevalence of AR exposure was lower in scavengers, some of them (10%) displayed high levels of summed SGARs. The AR exposure pathway for scavengers is not obvious. In the areas of carcass collection, reintroduced scavengers forage at feeding stations stocked with dead livestock by local farmers and with butchery remains (including bones for Bearded vultures) by field agents. While accidental primary AR exposure of domestic ruminants can occur (e.g., recently described poisoning event in ewes) (Moriceau et al., 2020) based on the sources of food and vigilance, substantial AR exposure at such supplemental feeding "vulture restaurants" seems unlikely. As vulture populations are growing in the area studied, feeding stations may not provide adequate food resources, resulting in some birds feeding on wild carrion. Presence of numerous illegal hunting "dumps" have been reported in the study area (Florence Buronfosse, National Centre for Veterinary Toxicological Information [CNITV], oral communication, 2021), and scavenging of the remains of large wild hunted mammals (e.g., boars) that may have consumed AR baits (Alabau et al., 2020) could be a source of exposure. This could especially be the case for Griffon vultures that are keen on viscera. Rodents might occasionally be part of the diet of Cinereous vultures (Xiao-Ti, 1991), and of young Bearded vultures, when food resources are scarce (Florence Buronfosse, National Centre for Veterinary Toxicological Information [CNITV], oral communication, 2021). Further investigation of feeding habits of vultures in the study area are needed to better understand AR exposure pathways.

4.2.2.2. Acute vs chronic multiple exposure. Less than 10% of positive samples displayed a concentration of an individual SGAR above the commonly used threshold of 100 ng/g ww, and none of the sampled birds presented evidence of AR toxicosis, suggesting that massive acute exposure was uncommon in raptors in our study (López-Perea and Mateo, 2018). The fact that high concentrations of a single ARs are rarely found could also suggest that much of the ingested AR is quickly eliminated, with only a small fraction persisting (Rached et al., 2020). This deserves further investigation through toxicokinetic studies.

In contrast, all birds with summed SGARs above 100 ng/g ww had multiple AR residues (i.e. up to 4 SGARs) and more than half of positive samples had residues of 2 or more ARs. This is consistent with other publications (Albert et al., 2010; Stone et al., 1999; Thomas et al., 2011), and suggests that chronic repeated exposure is a more prevalent pathway of exposure in wild birds, which can lead to sublethal adverse effects (Rattner and Harvey, 2021).

4.2.2.3. Other factors affecting AR exposure. Sex ratio of adult bird populations, including raptors, has received limited attention, but there is some evidence that in free-ranging populations males outnumber females (Donald, 2007). Notably, for the Andean condor (*Vulture gryphus*), an age-dependent skewed sex ratio favoring mature males has been described, with a proposed explanation that immature females forage in plains which might enhance exposure to human threats such as environmental contaminants (Lambertucci et al., 2012). However, in the present study, involving many different species of raptors, no such sex-based pattern of AR exposure was apparent, which is generally similar to observations in another AR exposure study (Murray, 2011).

While human population density, urbanization, density of livestock and acreage of crops have been observed to influence the magnitude

Table 3
Levels of ARs, haemorrhagic lesions and presence of blood clots in individuals suspected of AR toxicosis.

Bird	Species	Year	CHLORO	BROMA	BFC	DFC	DFT	∑SGARs	Macroscopic haemorrhagic lesions at necropsy	Blood clots in cardiac cavity
			(ng/g ww)							(Y/N/NA)
1	Red kite	2013	0	31.6	2.1	0	0	33.7	Subcutaneous hematoma of the thoracic area Serohaemorrhagic pericardial effusion	Y
2	Red kite	2018	0	2.1	25.2	0	0	27.3	Haemorrhage of the right eye Slight bleeding of lung surface	Y
3	Red kite	2018	0	52.5	8	63.2	68.2	191.9	Hematoma of right pectoral muscle and neck Blood in abdominal cavity Lung haemorrhage Haemorrhage of digestive tract Several haematoma on the surface of the skull bone	N
4	Red kite	2019	0	4.4	86	0	5.8	96.2	Subcutaneous hematoma of the thoracic area Lung haemorrhage Haemorrhage of pericardium	N

Abbreviations: chlorophacinone, CHLORO; bromadiolone, BROMA; brodifacoum, BFC; difenacoum, DFC; difethialone, DFT; second-generation anticoagulant rodenticide, SGAR.

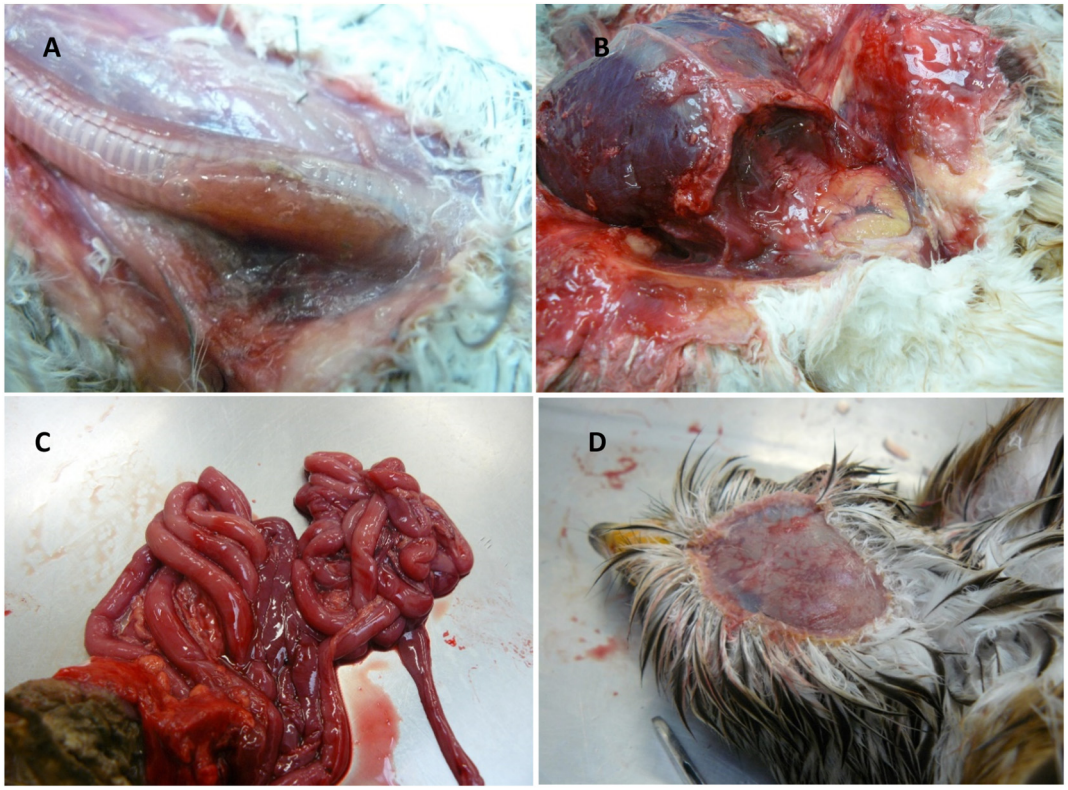

Fig. 5. Haemorrhagic lesions in bird n°3. A: Cervical haematoma, surrounding the trachea - B: Blood in coelomic cavity - C: Haemorrhage of digestive tract- D: Haematoma on the surface of the skull.

of SGAR exposure (Coeurdassier et al., 2019; López-Perea et al., 2019), no geographic trend for scavenging or predatory birds was apparent in the present study. This is likely a deficiency of recordkeeping for habitat type where carcasses were discovered (e.g., urban, suburban, agriculture, rural, natural area) could be addressed in this monitoring scheme, using available public data on land use.

4.3. Effect of public policies on AR exposure

Following the restriction of chlorophacinone use as a PPP in agricultural settings at the end of 2010, the frequency of detection in liver of raptors decreased. However, this trend should be viewed with caution as it is based on few detections. Furthermore, chlorophacinone is seemingly less persistent than several SGARs in liver (Vandenbroucke et al., 2008) which may limit its probability of detection. Bromadiolone was the most frequently detected SGAR in this study, and it may partly be explained by the fact that between 2010 and 2020 it was the only AR authorized in France as a PPP. Continued monitoring of bromadiolone since its 2020 withdrawal as a PPP would reveal how frequency of detection and residue concentrations change over time in non-target raptors.

While from 2013, use of bait stations was made mandatory for ARs used as biocides (Berny et al., 2014; Nakagawa et al., 2015; Suárez and Cueto, 2018), the trend observed in the present study seemed to be an increase in the frequency of detection of SGARs (Fig. 2), with this being significant for brodifacoum. These findings suggest that further measures may need to be considered to mitigate the risk of exposure in non-target fauna to SGARs used as biocides. It also suggests that exposure to PPP in agricultural settings is not the only pathway for AR exposure of wild birds, especially considering that their use is currently banned in the EU (EU Pesticides database, https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database_en, access on 14 October 2021); use of AR biocides in urban areas and cattle farms could even be the principal exposure route for wildlife (López-Perea et al., 2019). Such an increase in the use of SGARs might be related to the occurrence of resistance to ARs in rodents pest species (Blažić et al., 2018; Boitet et al., 2018; Buckle et al., 1994; Lefebvre et al., 2016; Marquez et al., 2019; McGee et al., 2020), and to the absence of efficient alternatives for pest control. Differences in potency and toxicity between stereoisomers of SGARs could be a promising direction for investigation to develop effective and more “eco-friendly” rodenticides (Damin-Pernik et al., 2016; Fourel et al., 2017b, 2021; Lefebvre et al., 2020).

4.4. Diagnosis of AR toxicosis

While many birds were exposed to ARs, toxicosis was suspected in only 2 of 156 individuals evaluated for rodenticide residues (i.e., 1.3% of sampled birds, 2.6% of birds with detectable AR residues). Suspicion of AR toxicosis in those 2 birds was established as they displayed macroscopic haemorrhage and clearly elevated AR residues (i.e., 96.2 and 191.9 ng summed SGARs/g ww) (Murray, 2018), and no other obvious cause of death was described in necropsy records. Using these criteria, 2 other birds might be suspected of succumbing from AR toxicosis (Table 3), yet residue concentrations were low and signs of haemorrhage were less apparent, and clotted blood was observed in the cardiac cavities of both individuals. This rate of toxicosis is consistent with prevalence reported in a recent study in Bald and Golden eagles (4% of eagles tested actually dying from AR toxicosis, Niedringhaus et al., 2021), suggesting that mortality directly related to AR consumption is rather low in non-target birds of prey. However, other studies in the northeastern United States involving several species of raptors indicate a greater frequency of AR toxicosis, with incidence of exposure increasing in recent years (Murray, 2011, 2017, 2020).

In contrast to these few suspected cases of AR toxicosis, the presence of haemorrhage was observed in a high proportion of birds (88/130, 67.7%), but most (71/88) were diagnosed with causes of death that result in haemorrhage (e.g., trauma, electrocution, infectious processes). No excessive bleeding from minor wounds was documented in any of

the cases, that might be suggestive of impaired coagulation (Murray, 2018). While some publications may suggest increased susceptibility to trauma in AR-intoxicated animals (Elmeros et al., 2011; Fournier-Chambrillon et al., 2004) no relationship was apparent between levels of AR exposure and presence of haemorrhage in birds that were classified as dying from trauma.

Our observations illustrate the difficulty of diagnosing AR toxicosis during post-mortem examination, especially for heterogeneous samples of wildlife with a high prevalence of other causes of death involving haemorrhage. No histopathological examination was performed on the samples to detect microscopic evidence of haemorrhage or hypoxic damage to tissues, as suggested by several previous studies (Murray, 2011, 2018; Niedringhaus et al., 2021; Rattner et al., 2011). However, this type of examination is not appropriate if carcasses are not fresh. Moreover, the codification of the presence/absence of haemorrhage was based on the personal interpretation of the senior author's review of descriptive data in each necropsy report based on keywords (i.e., "haemorrhage", "bleeding", "anaemia","bruising", "haematoma","blood"). Efforts are underway to improve the coding of necropsy observations in our internal wildlife mortality database.

To assist with diagnosis of AR toxicosis, many diagnosticians were led to the implementation of a toxicity threshold for summed SGARs, with a commonly reported threshold of 100 ng/g ww (Newton et al., 1990). However, in the present dataset this threshold concentration did not seem to reveal many individuals that died from AR toxicosis. In addition, no significant difference could be found when excluding potentially confounding factors (e.g., trauma and electrocution as cause of death) or by the grouping of samples by species to account for potential species-specific differences in AR sensitivity that limit the utility of this threshold (Rattner and Harvey, 2021). Drawbacks of the present study and dataset that limit our availability to examine the AR toxicity threshold for wild birds include small sample size for many of the species, and unlike other studies (e.g., Thomas et al., 2011; Murray, 2020), few individuals which seemingly died from AR toxicosis sequelae including coagulopathy. Moreover, carcasses could be more or less decomposed, possibly affecting AR concentration estimates in sampled livers (Valverde et al., 2020). It is noteworthy that some have suggested that such a residue threshold may not be a relevant diagnostic tool for AR toxicosis, as it does not address sublethal effects and the potential consequences of bioaccumulation from repeated exposures (Erickson and Urban, 2004; Rattner and Harvey, 2021). Development of additional biomarkers for AR effects could be useful in monitoring the effect of rodenticides on non-target fauna (Rached et al., 2020).

4.5. Concomitant exposure to other contaminants

In our study, most of the samples displaying elevated Pb exposure (>6 μg/g dw) were also positive for SGARs. The majority of those birds with exposure to both AR and Pb died from either trauma or electrocution. Combined sequential exposure to both lead and AR could have increased the risk of mortality in those birds, as these contaminants may impair flight coordination and/or spatial recognition (Herring and Eagles-Smith, 2017; Murray, 2011; Rattner et al., 2011). Furthermore, through different mechanisms of action, both lead and ARs can cause anaemia (Rattner and Mastrota, 2018). Further investigations are needed to characterise such potential "cocktail effects" of environmental contaminants commonly encountered by wild birds.

5. Conclusion

This study aimed at characterizing exposure to ARs in a large yet heterogeneous sample of birds of prey from southeastern France collected over a 12 year period. Results were generally consistent with other reports, documenting a rather high prevalence of AR exposure (mainly SGARs), usually low levels of individual ARs and summed of SGARs, and occasionally with a few birds seemingly succumbing to AR toxicosis. Multiple exposure was quite frequent, raising the issue of sub-lethal chronic effects related to repeated exposure. As expected, levels of ARs were higher in predators, but some scavengers also displayed high levels for which a pathway of exposure could not be fully elucidated. It was also noted that current public policies had a limited effect on mitigating AR exposure; the development of more eco-friendly ARs could be an approach to limit AR contamination of non-target fauna. The study also confirmed that diagnosis of AR toxicosis is a complex issue, and establishing a threshold like the widely used value of 100 ng/g ww may not be ideal. Future research exploring biomarkers of effect rather than just exposure, could permit discrimination of AR exposure and toxicosis. The presence of both ARs and Pb in some samples demonstrates multiple contaminant exposures that could have the potential to result in additive or synergistic effects.

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CRediT authorship contribution statement

Conceptualization: SL, IF, EB, FB, BAR, VL
Formal analysis: MAM, IF, SL, VL
Funding acquisition: FB, PO, EB, VL
Investigation: MAM, IF, FB, PO, VL
Methodology: MAM, SL, IF, FB, BAR, VL
Project administration: PO, FB, VL
Resources: PO, FB, VL
Supervision and Validation: VL BAR;
Writing original draft: MAM, BAR, VL
Review&Editing: MAM, SL, IF, EB, FB, PO, BAR, VL

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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