Received: 11 July 2023, Accepted: 28 August 2023

Review Article

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 8, 1709-1716

Anticoagulant Rodenticides in Nocturnal Birds of Prey: A European Perspective

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Abstract

Anticoagulant rodenticides are biocides that interfere with normal blood clotting, inhibit the vitamin K cycle in the liver, and cause death by hemorrhages. Even though the main target of these compounds is rodents, they may affect non-target species such as nocturnal birds of prey that feed on those rodents. To study secondary exposure to ARs, select species that specialize in rodent prey, such as nocturnal birds of prey. Besides their specialized diet in rodents, nocturnal birds of prey are one of the most widely distributed birds in Europe and live in rural and non-rural habitats, making them excellent sentinel species for several studies' ecotoxicology studies. There are numerous studies regarding secondary AR exposure in raptor species all around the world, but evidence for population-level effects is still absent. The objective of this review is to show how ARs have influenced wild nocturnal birds of prey in Europe in the last decades, most affected species, and in summary, explain how they act and the main clinical signals/ lesions that can be observed in poisoned birds. Overall, a total of 19 works were included in this review, between the years 1983 to 2021 that satisfied all literature criteria. These 19 papers corresponded to 44 observations of different species, regarding eight types of anticoagulant rodenticide. In the future, more caution is needed in the use of anticoagulants for rodent control where avian predators may be exposed to poisoned prey. Some combinations can be highly lethal to the predator, putting it at risk species that are already treated, therefore new eco-friendly alternatives should be found.

KEYWORDS
Rodenticides, Birds' prey, Nocturnal, Poisoning, Mouse, Mortality.

Introduction

Rodenticides are the most available pest control worldwide for rodents. They are used commercially to protect crops and stored food from these pests (Langford *et al*., 2013). The first anticoagulant rodenticides (ARs) appeared in the 1940s (van den Brink *et al*., 2018). ARs are chemical products that interfere with normal blood clotting, inhibit the vitamin K cycle in the liver, and cause death by haemorrhages (Lambert *et al*., 2007; Ruiz-Suárez *et al*., 2015). They can be divided into first and second-generation substances (López-Perea and Mateo, 2018).

Even though the main target of these biocides is rodents, they may affect non-target species, either by direct consumption of contaminated cereal-based baits or indirectly by consumption of contaminated prey (secondary poisoning) (Lambert *et al*., 2007). Monitoring programs of toxicovigilance have revealed the negative environmental consequences of ARs in non-target species such as birds and mammals. The half-life of ARs in the liver can differ between 0.3 to 66.8 days for first-generation rodenticides and 28.1 to 350 days for second-generation rodenticides (Lambert, 1997; Lambert *et al*., 2007). To study secondary exposure to ARs it is necessary to select species that focus on rodent prey, such as nocturnal birds of prey. Besides their specialized diet in rodents, nocturnal birds of prey are one of the most widely distributed birds in Europe and live in rural and non-rural habitats, making them excellent sentinel species for several ecotoxicology studies (Gray *et al*., 1994; Walker *et al*., 2008).

There are numerous studies regarding secondary AR exposure in raptor species all around the world, but evidence for population-level effects is still absent (Gomez *et al*., 2021). The objective of this review was to show how ARs have influenced wild nocturnal birds of prey in Europe in the last decades, the most affected species, and in summary, explain how they act and the main clinical signals/ lesions that can be observed in poisoned birds.

Types of anticoagulants rodenticides and mechanisms of action

Anticoagulant rodenticides are extensively used as biocides in agricultural and urban sites to control populations of rodents (rats, mice, voles) (Geduhn *et al*., 2015; López-Perea and Mateo, 2018). They are divided into two 'generations' based simply on when they were introduced in the market and their toxicity. First-generation AR was first introduced in the 1940s and was constituted by Warfarin, Coumatetralyl and Chlorophacinone (Ruiz-Suárez *et al*., 2015). The second generation of AR was introduced later in the 1970s, due to the increasing resistance to the older generation of AR (Walker *et al*., 2008), and includes compounds such as Difenacoum, Bromadiolone, Brodifacoum,

ISSN: 2090-6277/2090-6269/

Flocoumafen, and Difethialone. The second generation is 100 to 1000 times more toxic than the first generation (Ruiz-Suárez *et al*., 2015). Also, they are more persistent and bioaccumulative (Koivisto *et al*., 2018). The enhanced toxicity AR is based on their higher binding affinity to the target enzyme VKOR in the liver of the animal, which leads to an extended retention time in the animal body (Hohenberger *et al*., 2022).

Their commercial formula is mainly wax baits, coated wheat baits, and gels that may be installed in bait boxes, in burrows or buried underground within rodent galleries (Broughton *et al*., 2022). The application of these biocides can be made throughout the year or in specific periods when pests are most abundant (Lambert *et al*., 2027).

Most rodenticides are anti-coagulants, which are chemical products that interfere with normal blood clotting (Ruiz-Suárez *et al*., 2015). They are easily absorbed through ingestion in the gastrointestinal tract and lead to the inhibition of vitamin K epoxide reductase. This enzymatic inhibition blocks vitamin K regeneration, and consequently, the vitamin K-dependent coagulation factors II, VII, IX and X are incorrectly synthesized and do not bear the post-translational carboxylation required for activation (Nakayama *et al*., 2019). This impairs normal blood coagulation and predisposes the animal to death by inducing diffuse haemorrhages (van den Brink *et al*., 2018; López and Múrcia, 2008; Ruiz-Suárez *et al*., 2015).

AR is regulated under the EU Biocides Directive and Plant Protection Products (PPPs). The Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) is responsible for placing on the market and regulating the use of biocidal products in Europe (European Chemical Agency (ECHA, 2023). In Europe, Eight ARs are currently registered for use (López-Perea and Mateo, 2018). One of the consequences of the use of AR is that non-target small mammal species can also take poisoned baits. Predators can be secondarily poisoned by ARs mainly as a result of preying on rodents and/or scavenging (Elliott *et al*., 2014; López-Perea and Mateo, 2018). This risk t is accredited in the assessment reports which recommend their inclusion in Annex I of the Biocides Directive (98/8/EC) and subsequent use in European Union member states (Hughes *et al*., 2013; Eisemann *et al*., 2018). Some ARs pose an enormous risk to non-target species, but due to the lack of alternatives, they can be used with some restrictions (limit to professional use only, targeted baiting, protective bait boxes, and removal of dead rodents) (Hughes *et al*., 2013).

Some rodent populations also have developed resistance to Difenacoum and Bromadiolone (second generation). The first (ECHA, 2023) cases have been detected in the UK and Norway (Buckle, 2013). This increasing resistance is an enormous problem. It is accelerated by the overuse of AR and by the misuse of compounds that are stronger than needed. The animals survive and reproduce, producing young offspring with resistance (Valverde *et al*., 2021).

Nocturnal bird of prey as bioindicators of environmental health

In the study of secondary exposure to ARs was necessary to select species specialization on rodent prey (Lambert *et al*., 2007; Ruiz-Suárez *et al*., 2015). Other important traits are limited territory distribution, restricted habitat utilization, partial migration, and lack of preference for mammalian prey (specialization in small mammals, mainly rodents). Applying these criteria, some of the most suitable species in Europe to use in these studies were the Tawny Owl, Barn Owl, Eurasian Eagle Owl, Little Owl, and Long-Eared Owl (Fig. 1) (Langford *et al*, 2013).

Fig. 1. From left to right Eurasian eagle owl (*Bubo bubo*), Barn Owl (*Tyto alba*) and Tawny Owl (*Strix aluco*) (Illustration Andreia Garcês).

There are a few characteristics that make nocturnal birds of prey excellent sentinels of environmental health, and ecotoxicological research. These include foraging through both terrestrial and aquatic food webs (small mammals, reptiles and amphibians), being very adaptable to urban and non-urban environments, occupying high positions in the trophic chain (apex predators), easy to obtain non-destructive samples such as feathers, carcasses from accidents, deserted eggs, or blood for analysis (Badry *et al*., 2020; 2022).

The Tawny Owl and barn owl are the species that have been used in both short and long-term monitoring of AR exposure in Europe (López-Perea and Mateo, 2018). Both species are very abundant in all European territories and are often victims of traffic collisions, therefore their carcasses are easily available for collection and analysis (Walker *et al*., 2008). However, Barn owls can be more restricted than the tawny owl in habitat use, tend to be found predominantly in agricultural landscapes, and are absent from parts of Europe, which can be a limitation. The Eurasian ea-

Table 1. List of traits from the different species of owl. Adapted from Badry *et al*. (2020)

Specie	Distribution	Diet	Habitat	Migration	Conservation status
Tawny owl (*Strix aluco*)	Eastern, Northern (except Ireland, Iceland), Southern and Western Europe	Small mammals' insects, birds	Urban habitats, farmland with patched forest	Resident	Least concern
Barn owl (*Tyto alba*)	Eastern, Northern (except Fennoscandia and Estonia), Southern, and Western Europe	Mainly rodents	Urban habitats, farmland	Resident	Least Concern
Eurasian eagle owl (*Bubo bubo*)	Eastern, Northern (except UK, Ireland, and Iceland), southern and Western Europe	Mammals and avian prey	Forest patches, agricultural habitats, open habitats	Resident	Least concern
Little owl (*Athene noctua*)	Eastern, northern (except Fennoscandia, Ireland, and Estonia), southern, and western (except alpine regions) Europe	Small mammals and invertebrates	Open farmland habitats	Resident	Least concern
Long-eared owl (*Asio otus*)	Eastern, Northern, Southern, and Western Europe	Small mammals and birds	Forest patches and agroforestry	Partial migration in Fennoscandia	Least concern

gle owl, long-eared owl and little owl are not very used in these studies, because although they can be exposed to ARs they are absent from diverse areas (Badry *et al.,* 2020). Some studies have already shown that some owl populations have been declining in regions where AR have been used in large quantities (Christensen *et al.,* 2012; Newton *et al.,* 1990).

Table 2.1. Anticoagulant rodenticides in nocturnal birds of prey by country, specie, year, sample type, compounds, concentration, and percentage of incidence. (BRD brodifacoum, BRM bromadiolone, COU coumatetralyl, DIF difenacoum, FLO flocoumafen, WAR warfarin, CHO chlorophacinone, DIP diphacinone)

Country/regions	Specie	Year	Sample	Compound	Concentration	Percentage of incidence (%)	Ref.
Norway	Eagle owl (*Bubo bubo*)	2009-2011	Liver	FLO, difethialone, DIF, BRM, BRD	11 and 255 ng/g w.w.	50	Langford *et al*. (2013)
United Kingdom	Tawny owls (*Strix aluco*)	1990-1993, 2003-2005	Liver	DIF, BRM, FLO, BRD	>0.1 µg/g wet w.t.	19.2	Walker *et al*. (2008)
	Barn owls (*Tyto alba*)	2007-2008	Liver	DIF, BRM	0.031 µg/g w.w. to 0.727 µg/g w. w.	81	Walker *et al*. (2010)
	Barn owls (*Tyto alba*)	1987, 1983, 19889	NA	BRD	0.005 ppm	NA	SHAWYER, 1989; Stone *et al*. (1999)
	Barn owls (*Tyto alba*)	2006-2012	Liver	BRM, DIF, BRD	>100 ng/g w. w.	71	Shore *et al*. (2019)
	Barn owls (*Tyto alba*)	1983-1989	liver	DIF, BRD	0.005-0.0106 µg/g w.w., 0.019-0.515 µg/g w.w.,	10	Newton *et al*. (1990)
Scotland	Tawny owls (*Strix aluco*)	1987-2005	Liver	DIF, BRM, FLO, BRD	NA	18.5	Walker *et al*. (2008)
	Barn owls (*Tyto alba*)	2000-2010	Liver	BRD, BRM, COU, DIF, FLO, WAR, CHO, DIP	0.048mg/kg	34.9	Hughes *et al*. (2013)
	Tawny owls (*Strix aluco*)	2000-2010	Liver	BRD, BRM, COU, DIF, FLO, WAR, CHO, DIP	0.029mg/kg	38.2	Hughes *et al*. (2013)
Spain	Tawny owls (*Strix aluco*)	2005-2010	Liver	BRD, BRM	0.028 and 0.008 µg/g w.w.	38.7	Sánchez-Barbudo *et al*. (2012)
	Eagle owl (*Bubo bubo*)	2005-2010	Liver	BRD, BRM, FLO	0.116, 0.004 and 0.011 µg/g w.w.,	38.7	Sánchez-Barbudo *et al*. (2012)
	Little Owl (*Athena noctua*)	2005-2010	Liver	DIF	0.056 µg/g w.w.,	38.7	Sánchez-Barbudo *et al*. (2012)
	Eagle owl (*Bubo bubo*)	2007-2016	Liver	BRM, DIF, FLO	279 ng/g w.w.	63	López-Perea *et al*. (2019)
	Little Owl (*Athena noctua*)	2007-2016	Liver	BRM, DIF, FLO	56 ng/g w.w.	100	López-Perea *et al*. (2019)
	Barn owls (*Tyto alba*)	2007-2016	Liver	BRM, DIF, FLO	53 ng/g w.w.	60	López-Perea *et al*. (2019)
	Scopus owl (*Otus scops*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	14.3	Ruiz-Suárez *et al*. (2015)
	Barn owl (*Tyto alba*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	54.5	Ruiz-Suárez *et al*. (2015)
	Tawny owl (*Strix aluco*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	77.8	Ruiz-Suárez *et al*. (2015)
	Eagle owl (*Bubo bubo*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	100	Ruiz-Suárez *et al*. (2015)
	Long-eared owl (*Asio otus*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	58.3	Ruiz-Suárez *et al*. (2015)
	Little owl (*Athene noctua*)	2011-2013	Liver	BRM, DIF, FLO, BRD	>200 ng/g	71.4	Ruiz-Suárez *et al*. (2015)
Canary Islands, Spain	Short eared owl (*Asio flammeus*)	2009-2012	Liver	BRM, BRD, DIF, CHO	77.2, 15.8, 2.9, 0.5 µg/g w.w.,	73.9	Ruiz-Suárez *et al*. (2015)
	Barn owls (*Tyto alba*)	2009-2012	Liver	BRM, BRD, DIF, CHO	75.8, 12.5, 12.6, 1.2 µg/g w.w.		Ruiz-Suárez *et al*. (2015)
Majorca Island, Spain	Scops owl (*Otus scops*)	2011-2013	Liver	BRM, DIF, BRD	>200 ng/g	57.7	Ruiz-Suárez *et al*. (2015)
	Barn owls (*Tyto alba*)	2011-2013	Liver	BRM, DIF, BRD	>200 ng/g	84.2	Ruiz-Suárez *et al*. (2015)
Estonian	Tawny owls (*Strix aluco*)	2013-2019	Liver	BRM, DIF, BRD	12, 0.2, 26.3 ng/g w. w.	NA	Peetris, 2019)
	Eagle owl (*Bubo bubo*)	2013-2019	Liver	BRM, DIF, BRD	7.6, 0.7, 4.2 ng/g w. w.	NA	Peetris, 2019)
	Short eared owl (*Asio flammeus*)	2013-2019	Liver	BRM, BRD	14.2, 13.5 ng/g w. w.	NA	Peetris, 2019)
	Ural owl (*Strix uralensis*)	2013-2019	Liver	BRM, DIF, BRD	39.5, 0.3, 6.4 ng/g w. w.	NA	Peetris, 2019)

Table 2.2. Anticoagulant rodenticides in nocturnal birds of prey by country, specie, year, sample type, compounds, concentration, and percentage of incidence. (BRD brodifacoum, BRM bromadiolone, COU coumatetralyl, DIF difenacoum, FLO flocoumafen, WAR warfarin, CHO chlorophacinone, DIP diphacinone)

Country/regions	Specie	Year	Sample	Compound	Concentration	Percentage of incidence (%)	Ref.
Denmark	Barn owl (*Tyto alba*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO	71 ng/g w.w.	94	Christensen *et al.* (2012)
	Eagle owl (*Bubo bubo*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO	241 ng/g w.w.	100	Christensen *et al.* (2012)
	Little owl (*Athena noctua*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO	39.0 ng/g w.w.	100	Christensen *et al.*, (2012)
	Long-eared owl (*Asio otus*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO	13.5 ng/g w.w.	95	Christensen *et al.*, (2012)
	Short-eared owl (*Asio flammeus*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO	18.0 ng/g w.w.	100	Christensen *et al.* (2012)
	Tawny owls (*Strix aluco*)	2000-2009	Liver	BRD, BRM, COU, DIF, FLO,	39.0 ng/g w.w.	93	Christensen *et al.* (2012)
France	Barn owls (*Tyto alba*)	2003	Liver	BRM, DIF	‡ 0.08 ug/g and < 0.25 ug/g	NA	Lambert *et al.* 2007)
	Tawny owls (*Strix aluco*)	2003	Liver	BRM, DIF	‡ 0.08 ug/g and < 0.25 ug/g	NA	Lambert *et al.* (2007)
Portugal	Barn owls (*Tyto alba*	2015-2016	Liver	BRM, DIF	NA	71,5	Marques (2017)
Ireland	Barn owls (*Tyto alba)*	1988-1989	Pellets	BRD, DIF, FLO	0.01-0-02 mg kg^{1}	97	Eadsforth *et al.* (1991)
Finland	Eagle owl (*Bubo bubo*)	2004-2014	Liver	COU, DIF, BRD, BRM, FLO	≥1 µg/kg	82	Koivisto *et al.* (2018)
	Tawny owls (*Strix aluco*)	2004-2014	Liver	COU, DIF, BRD, BRM, FLO	≥1 µg/kg	82	Koivisto *et al.* (2018)
Germany	Barn owls (*Tyto alba*	2011-2013	Pellet, liver	BRD, BRM, DIF, FLO, COU, WAR.	NA	1 pellet, 55 liver	Elliott *et al.* (2014)

Secondary anticoagulant rodenticide intoxications in birds

Rodents can survive for several days (generally 2-4 days) after consuming a lethal dose of ARs and often will continue feeding on the bait for several days (Thomas *et al.*, 2011). During this period the rodents remain active and can be captured by preda tors. They can have erratic behaviors, such as spending more time in open areas in a lethargic state or reduced censorial capacity, which predisposes them to predation (Thomas *et al.*, 2011; Ruiz-Suárez *et al.*, 2015;). During the period when rodents feed baits they consume up to 8-10 times the LD50 of the products used (Ruiz-Suárez *et al.*, 2015), therefore they can have a concentration in their body that exceeds the LD50 or even LD100 dose (Thomas *et al.*, 2011; Langford *et al.*, 2013). A rodent can also ingest a sub-lethal dose and may carry the toxic in its liver for several months (Ruiz-Suárez *et al.*, 2015).

Nocturnal raptors can feed on rodents poisoned with ARs but also feed on granivorous birds that sometimes have accidentally ingested cereal baits When raptors consume this poisoned prey ARs residues accumulate in the tissues and in many cases, this exposure can lead the birds to secondary poisoning that can make them weaken or even lead to death (Ruiz-Suárez *et al.*, 2015). Figures 2 and 3 represent a conceptual model of ecotoxicology in rodents (Dodds-Smith *et al.*, 1992). Secondary poisoning can occur via target and non-target small mammals. The main target species of AR applications in Europe are Rattus norvegicus and *Mus musculus* (Geduhn *et al.*, 2015). Studies in Barn Owls show that it takes 6 to 17 days to die after consuming 3 mice containing the poison Brodifacoum (van den Brink *et al.*, 2018a). In birds, ARs can bind and inhibit vitamin K epoxide reductase and can persist for at least six months in organs and tissues containing this enzyme such as the liver (Thomas *et al.*, 2011).

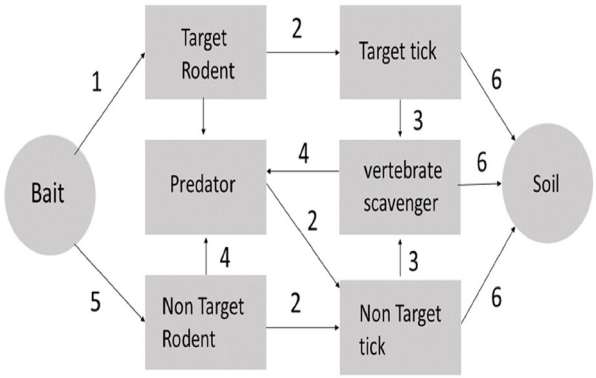

Fig. 2. Conceptual model of ecotoxicology in rodents. 1 – Primary ingestion of bait by tar-get rodents; 2 – Mortality due to the primary and secondary poisoning of target and non-target individuals; 3 – Carcasses ingestion by vertebrate scavengers; 4 – Predation of target and non-target vertebrates; 5 – Primary ingestion of bait by non-target rodents; 6 – Xenobiotic transference from the carcasses to the soil.

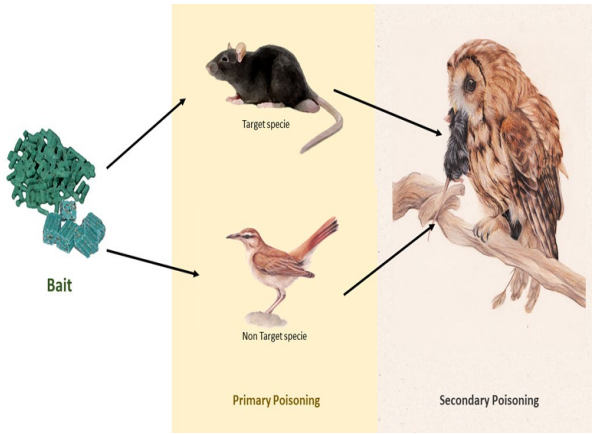

Fig.3. Conceptual model of ecotoxicology in rodents and nocturnal birds of prey (Illustration Andreia Garcês)..

Anticoagulant rodenticide consequences in birds

In birds, it is possible to observe clinical signals and macro/microscopic lesions associated with secondary poisoning by ARs. The severity of these signs is dose-dependent, and they can only appear a few days or weeks following AR exposure (van den Brink *et al.*, 2018).

Macroscopic lesions

Some of the lesions observed in poisoned birds are bruising, haemorrhage (e.g., skin, alimentary tract, peritoneal cavity, kidney, liver), blood loss from the oral cavity, nares, cloaca, and talons, and blood in scat (Fig. 4) (van den Brink *et al.*, 2018). Bleeding can also occur in the body cavity, under the skin, or in muscles.

Haemorrhages often extend over a considerable area of the bird's body, for example along the entirety of the pectoral muscles, or across the abdominal wall. Importantly, these haemorrhages occur without simultaneous signs of trauma such as fractured bones or lacerations (Fig. 4) (van den Brink *et al.*, 2018).

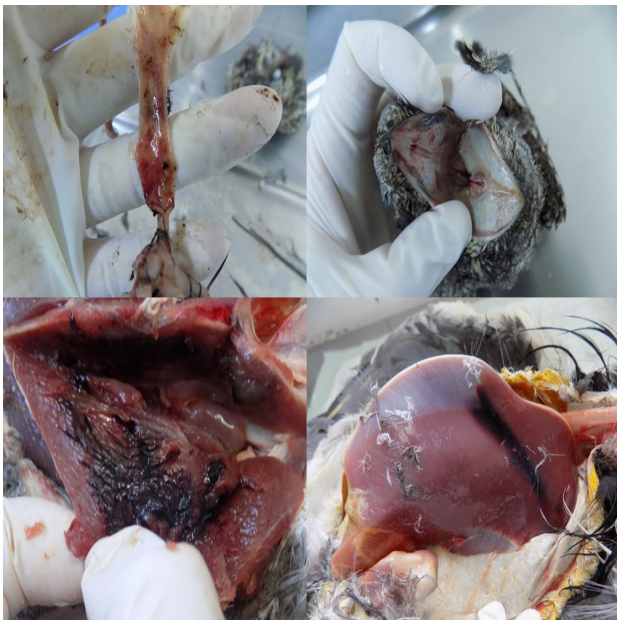

Fig. 4. Haemorrhages in the gastrointestinal tract (upper image) and oral cavity of birds and haemorrhages in the pectoral muscle of birds (down image) (Author Andreia Garcês).

It is important to distinguish traumatic bruising from haemorrhage from AR toxicosis. In case of trauma the localized bruising is associated with injury, but generally will not be accompanied by severe pallor of internal organs. When occurs AR toxicosis is possible to observe extensive bruising over multiple regions of the body, no associated fractures, a large amount of blood loss associated with small wounds, a large amount of blood in the body cavity, and pallor of internal organs (van den Brink *et al.*, 2018).

Severe haemorrhage and hypovolemic shock lead to depressed mentation (lethargy, quiet behaviour, closing of the eyes, unresponsiveness), weakness, and pallor of the mucous membranes (Fig. 5). These are the most clinical signs observed in AR-intoxicated birds, and are caused by decreased oxygen delivery to tissue, decreased blood pressure secondary to blood loss, and peripheral vasoconstriction. Therefore, is very common for animals to exhibit decreased hematocrit (Hct - packed cell volume) clinically classified as anaemia due to blood loss (Broughton *et al.*, 2022). The Hct is often less than 20% (normal values are between 35-45%), and there have been cases where was observed as low as 6% (Colvin and Hegdal, 1988; Gray *et al.*, 1994; van den Brink *et al.*, 2018).

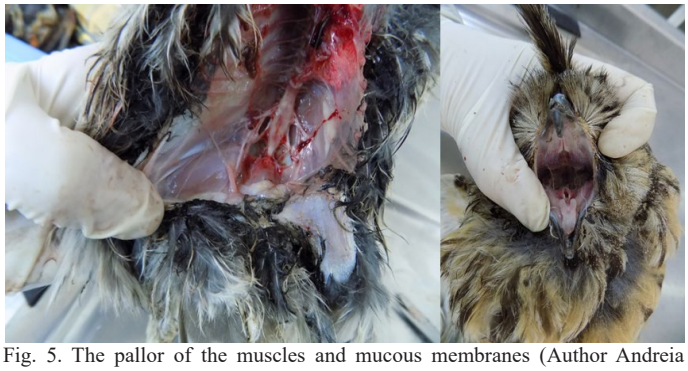

Fig. 5. The pallor of the muscles and mucous membranes (Author Andreia Garcês).

Death due to AR intoxication may result from exsanguination or hypoxia and multi-organ failure. The presence of blood in large quantities in or around critical organs (e.g., the brain, heart, and lungs) can also lead to the appearance of clinical signs and death (Geduhn *et al.*, 2015; van den Brink *et al.*, 2018).

Microscopic lesions

Histological observations alone cannot provide a final diagnosis of AR intoxication (van den Brink *et al.*, 2018). It is possible to observe haemorrhage in multiple tissues (heart, lung, kidney, liver and skeletal muscle), and hypoxic damage to organs. In some cases, tissue necrosis has been reported (van den Brink *et al.*, 2018; Gomez *et al.*, 2021) (Fig. 6).

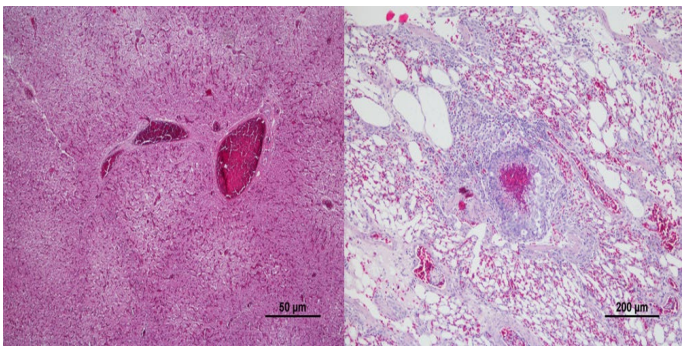

Fig. 6. Haemorrhages in the liver and lung of birds (left to right) (Author Andreia Garcês).

Sublethal effects

Exposure to ARs by nocturnal birds of prey in most cases is not sufficient to kill the animals immediately but can cause sub-lethal effects that may impair the fitness of individuals. Anorexia, lethargy, reduced agility, susceptibility to disease, reduced resilience or tolerance to extreme weather, and wing dropping are common to observe. The animals also can present an increase in the coagulation time measured (i.e., thrombin time, prothrombin time, activated partial thromboplastin time) (van den Brink *et al.*, 2018; López-Perea and Mateo, 2018).

Sub-lethal adverse effects of AR poisoning may occur not only at the individual level but may also disturb population dynamics. For example, reproduction can be affected. Studies have shown evidence of adverse effects of compounds such as ARs on the breeding success of secondary poisoning animals (van den Brink *et al.*, 2018; Gabriel *et al.*, 2018; Newton *et al.*, 1990). Also, there is the risk of maternal transfer of ARs to the progeny (van den Brink *et al.*, 2018). Some effects also may manifest on a molecular and cellular level, as vitamin K is needed for genetic pro-

cesses including RNA transcription and xenobiotic metabolism (Gomez *et al*., 2021).

Research studies on anticoagulant rodenticides in nocturnal birds of prey

The initial search identified 1100 articles from the databases - MEDLINE, Web of Science, ResearchGate, and Google Scholar. In the first showing of all abstracts, 800 articles were excluded, remaining 300. Of these 75 were repeated and were excluded. To the remaining 225 articles, a primary exclusion filter was applied: 101 were excluded due to geography (out of Europe), and three due to language. With secondary exclusion filters screening to full-review the articles: 98 were excluded since were not performed in wild animals and 4 were no open-access full articles (it was not possible to consult methods and results). Therefore, 19 articles were identified for a full review of the Systematic Review (Fig. 7) (Page *et al*., 2021) and are summarized in Table 2.

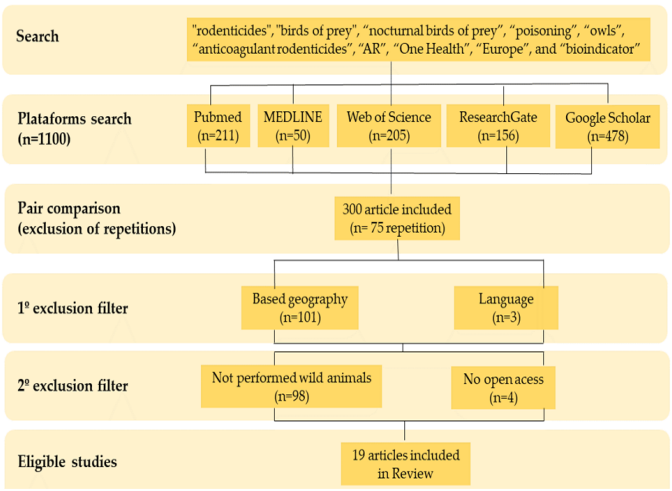

Fig. 7. Flow diagram of data collection for the review.

Species and spatial distribution

The main species where the studies were performed were: 31% (n=14) Barn owls (*Tyto alba*), 18% (n=8) Eagle owl (*Bubo bubo*), 18% (n=8) Tawny owls (*Strix aluco*), 7% (n=3) Little Owl (*Athena noctua*), 7% (n=3) Short-eared owl (*Asio flammeus*), 5% (n=2) Scopus owl (*Otus scops*), 5% (n=2) Long-eared owl (*Asio otus*), and 2% (n=1) Ural owl (*Strix uralensis*).

The countries where the studies were performed: 36% (n=16) Spain, 18% (n=8) United Kingdom, 14% (n=6) Denmark, 9% (n=4) Estonian, 5% (n=2) France, 5% (n=2) Finland, 2% (n=1) Portugal, 2% (n=1), Ireland, 2% (n=1) Norway, 2% (n=1) Germany (Fig. 8).

Compounds, type samples and concentrations

Almost every study was performed in the liver of dead animals, except that were performed in pellets of owls. The majority of samples where the compounds were analysed were liver. The most common compounds observed were brodifacoum, bromadiolone, coumatetralyl, difenacoum, and flocoumafen, a second-generation anticoagulant rodenticide (Table 2).

Effect of anticoagulant rodenticides on nocturnal birds of prey

In overall research about the issue, this appears to be the first systemic review on AR in wild nocturnal birds in Europe. The available bibliography appears scarce source and disperse on this

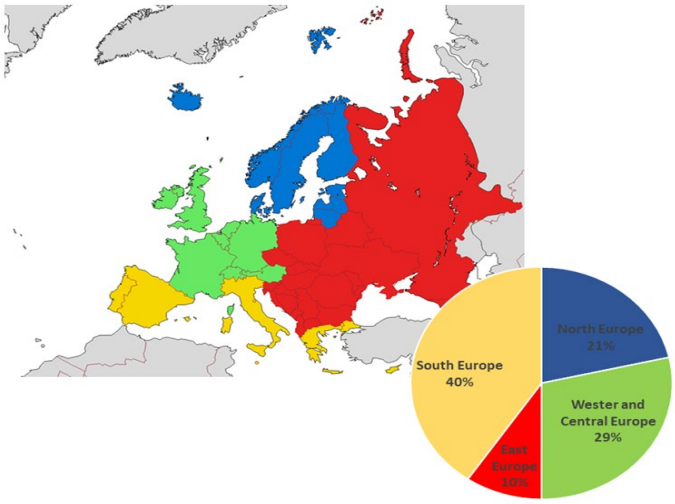

Fig. 8. Spatial distribution in the different regions of Europe.

subject, although there is a great interest in AR in non-target animals (Eadsforth *et al*., 1991; Dodds-Smith *et al*., 1992; Hadler and Buckle, 1992; Horak *et al*., 2018; Hughes *et al*., 2013; Koivisto *et al*., 2018). In the late 1970s, the first deaths associated with secondary poisoning by AR were reported in owls and other animals (Hohenberger *et al*., 2022).

The majority of the studies were performed on Barn owls (31%) (Gray *et al*., 1994; Fajardo, 2001; Shore *et al*., 2019). This species can be considered one of the best bio-indicators regarding toxicological studies on AR due to their preference for rodents, large distribution and limited migration habits (Newton *et al*., 1994; Salim *et al*., 2014).

The main compounds observed were brodifacoum, bromadiolone, coumatetralyl, DIF difenacoum, flocoumafen, warfarin, chlorophacinone, and diphacinone, from these, only warfarin and chlorophacinone are belong to the first-generation AR (Watt *et al*., 2005). This phenomenon was expected since the most used in the latest years have been secondary generation AR (Martínez-Padilla *et al*., 2017) which is more potent because rodents had acquired resistance to the first generation (Huang *et al*., 2022). The problem with second-generation AR is that they are more lethal to rodents but also have a higher risk of secondary poisoning for non-target species such as nocturnal birds or scavengers (Gabriel *et al*., 2018; Oliva-Vidal *et al*., 2022). The more potent AR can remain in the rodent's bodies for longer periods and accumulate, and therefore predators that consume these poisoned rodents will ingest higher amounts of AR and have a higher risk of developing poisoning symptoms (Watt *et al*., 2005; Hohenberger *et al*., 2022).

In these animals would be important the establishment of AR hepatic residue levels is associated with toxicity and lethality. Newton *et al*. (1999) suggested that the toxicity limits would be between 100–200 ng/g (w.w.) in the liver (Newton *et al*., 1990). Unfortunately, the sensitivity varies among species and individuals (López and Múr-cia, 2008). According to some studies, some species like the Great Horned Owl (Bubo virginianus) have a 5% probability of exhibiting clinical signs of toxicosis with AR liver residues of 20 ng/g (w.w.). In Eastern Screech Owls (*Megascops asio*) signs of coagulopathy were associated with liver diphacinone levels exceeding 100 ng/g (w.w.) (López and Múrcia, 2008). Some studies also suggest that older birds may be more at risk due to bioaccumulation (Elliott *et al*., 2014).

In addition to secondary poisoning, AR can have other effects on the population that are difficult to measure. The use of AR can increase the risk of pathogen infection among wildlife. Rodents that ingest ARs are weaker and have immune suppression, and

therefore are more susceptible to being infected with zoonotic pathogens such as Leptospira spp., Salmonella spp., and Hantavirus, among others. The rodents can pass these diseases to their predators/scavengers, but also transmit them to humans, leading to public health issues (Murray and Sánchez, 2021). Animals that have been poisoned, even in sublethal doses, also can be more susceptible to parasites infections and other diseases (Lemus *et al*., 2011)

For an AR to be authorized the product should be effective and have no effects on human health, animal health, and the environment (ECHA, 2023). According to Articles 5 and 19 of the BPR (Biocidal Products Regulation 528/2012) the baits containing ARs should have a lethal effect on the target organisms, not lead to the development of resistance, not be toxic, bioaccumulating, persistent, carcinogenic, mutagenic, or an endocrine disruptor, and always, if possible, to be formulated in the form of bait (Hohenberger *et al*., 2022; Watt *et al*., 2005). An eco-friendlier and more species-specific chemical alternative should be developed in the future, particularly when used in areas where threatened species inhabit (Lohr, 2018). In the future, these substances should be improved. Some of the improvements are for example: being designed specifically for the target organisms to avoid primary and secondary poisoning of non-target species, not leading to bait inhibition, knowing the action mode of the compound, killing humanely, economically efficiently, and having an antidote in case of accidental poisoning (Hohenberger *et al*., 2022)

Conclusion

Anticoagulant rodenticides have negative impacts on nocturnal birds of prey all around Europe. Although many studies have been performed there is information that is still missing regarding the impact of AR on the birds' populations. Population-level effects were to occur, and the impact on the ecosystem functions and services provided by raptors. The increased use and overuse of AR is a great problem in some regions particularly because rodents can become immune to their effect and bioaccumulate these compounds in their tissues, leading to the death or low performance of their predators. To the authors' knowledge, this is the first review on AR intoxication in free-living nocturnal birds of prey performed. Further research is needed to understand the connection between AR exposure and changes in population growth, survival rate, and reproductive success. Also, more studies are necessary for other parts of Europe to comprehend what measures can be implemented to help these populations and find new alternatives to AR.

Acknowledgments

The participation of Pires I was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020, funded by the Portuguese Foundation for Science and Technology (FCT). (Project UIDB/CVT/0772/2020). The participation of Garcês A. was supported by National Funds from FCT Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

REFERENCES

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: Identification of key species for pan-European biomonitoring. Sci. Total Environ. 731, 139198. https://doi.org/10.1016/j.scitotenv.2020.139198.

Badry, A., Schenke, D., Brücher, H., Chakarov, N., Grünkorn, T., Illner H, Krüger, O.; Marczak, T.; Müskens, G.; Nachtigall, W., 2022. Spatial variation of rodenticides and emerging contaminants in blood of raptor nestlings from Germany. Environ. Sci. Pollut Res. 29, 60908–21. https://doi.org/10.1007/s11356-022-20089-1.

van den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A., 2018 Anticoagulant Rodenticides and Wildlife. Vol. 5. Springer International Publishing. USA. https://doi.org/10.1007/978-3-319-64377-9.

Broughton, R.K., Searle, K.R., Walker, L.A., Potter, E.D., Pereira, M.G., Carter, H., Sleep, D.; Noble, D.G., Butler, A.; Johnson, A.C., 2022. Long-term trends of second-generation anticoagulant rodenticides (SGARs) show widespread contamination of a bird-eating predator, the Eurasian Sparrowhawk (Accipiter nisus) in Britain. Environ. Pollut. 314, 120269. https://doi.org/10.1016/j.envpol.2022.120269.

Buckle A., 2013. Anticoagulant resistance in the United Kingdom and a new guideline for the management of resistant infestations of Norway rats (Rattus norvegicus Berk.). Pest. Manag. Sci. 69, 334–341. https://doi.org/10.1002/ps.3309.

Christensen, T.K., Lassen, P., Elmeros, M., 2012. High Exposure Rates of Anticoagulant Rodenticides in Predatory Bird Species in Intensively Managed Landscapes in Denmark. Arch. Environ. Contam. Toxicol. 63, 437–44. https://doi.org/10.1007/s00244-012-9771-6.

Colvin, B.A., Hegdal, P.L., 1998. Procedures for Assessing Secondary Poisoning Hazards of Rodenticides to Owls. Vertebrate Pest Control and Management Materials: 5th Volume, ASTM International. ATM, USA. p. 64–71. https://doi.org/10.1520/STP26172S.

Dodds-Smith, M.E., Johnson, M.S., Thompson, D.J., 1992. Trace metal accumulation by the shrew Sorex araneus,: I. Total Body Burden, Growth, and MortalityEcotoxicol. Environ. Saf. 24, 102–117. https://doi.org/10.1016/0147-6513(92)90039-6.

Eadsforth, C.V., Dutton, A.J., Harrison, E.G., Vaughan, J.A., 1991. A barn owl feeding study with [14c]flocoumafen-dosed mice: Validation of a non-invasive method of monitoring exposure of barn owls to anticoagulant rodenticides in their prey. Pestic. Sci. 32, 105–119. https://doi.org/10.1002/ps.2780320111.

ECH, 2023. European Chemical Agency. https://echa.europa.eu (accessed 22 June 2023).

Eisemann, J.D., Fisher, P.M., Buckle, A., Humphrys, S., 2018. An International Perspective on the Regulation of Rodenticides. In: Anticoagulant Rodenticides and Wildlife. Vol 5, Springer International Publishing, USA, p. 287–318. https://doi.org/10.1007/978-3-319-64377-9_11.

Elliott, J.E., Hindmarch, S., Albert, C.A., Emery, J., Mineau, P., Maisonneuve, F., 2014. Exposure pathways of anticoagulant rodenticides to nontarget wildlife. Environ. Monit. Assess. 186, 895–906. https://doi.org/10.1007/s10661-013-3422-x.

Fajardo, I., 2001. Monitoring non-natural mortality in the barn owl (*Tyto alba*), as an indicator of land use and social awareness in Spain. Biol. Conserv. 97, 143–149. https://doi.org/10.1016/S0006-3207(00)00091-4.

Gabriel, M., Diller, L., Dumbacher, J., Wengert. G,, Higley. J,, Poppenga, R., Mendia, S., 2018. Exposure to rodenticides in Northern Spotted and Barred Owls on remote forest lands in northwestern California: evidence of food web contamination. Avian Conserv. Ecol. 13. https://doi.org/10.5751/ACE-01134-130102.

Geduhn, A., Jacob, J., Schenke, D., Keller, B., Kleinschmidt, S., Esther, A. 2015. Relation between Intensity of Biocide Practice and Residues of Anticoagulant Rodenticides in Red Foxes (Vulpes vulpes). PLoS ONE. 10, e0139191. https://doi.org/10.1371/journal.pone.0139191.

Gomez, E.A., Hindmarch, S., Smith, J.A., 2021. Conservation Letter: Raptors and Anticoagulant Rodenticides. J. Raptor Res. 56, 147–153. https://doi.org/10.3356/JRR-20-122.

Gray, A., Eadsforth, C.V., Dutton, A.J., Vaughan, J.A., 1994. Non-invasive method for monitoring the exposure of Barn owls to second-generation rodenticides. Pestic. Sci. 41:339–43. https://doi.org/10.1002/ps.2780410409.

Hadler, M.R., Buckle, A.P., 1992. Forty-five years of anticoagulant rodenticides--past, present and future trends. Proceedings of the Vertebrate Pest Conference Newport Beach, California, pp.15.

Hohenberger, J., Friesen, A., Wieck, S., Kümmerer, K., 2022. In search of the Holy Grail of Rodent control: Step-by-step implementation of safe and sustainable-by-design principles on the example of rodenticides. Sustain. Chem. Pharm. 25, 100602. https://doi.org/10.1016/j.scp.2022.100602.

Horak, K.E., Fisher, P.M., Hopkins, B., 2018. Pharmacokinetics of Anticoagulant Rodenticides in Target and Non-target Organisms. In Anticoagulant Rodenticides and Wildlife, Vol 5, Springer International Publishig, USA, pp. 87–108. https://doi.org/10.1007/978-3-319-64377-9_4.

Huang, E.Y.Y., Law, S.T.S., Nong, W., Yip, H.Y., Uea-Anuwong, T., Magouras, I., 2022. The screening for anticoagulant rodenticide gene VKORC1 polymorphism in the rat Rattus norvegicus, Rattus tanezumi and Rattus losea in Hong Kong. Sci Rep. 12,12545. https://doi.org/10.1038/s41598-022-16550-3.

Hughes, J., Sharp, E., Taylor, M.J., Melton, L., Hartley, G., 2013. Monitoring agricultural rodenticide use and secondary exposure of raptors in Scotland. Ecotoxicology. 22, 974–284. https://doi.org/10.1007/s10646-013-1074-9.

Koivisto, E., Santangeli, A., Koivisto, P., Korkolainen, T., Vuorisalo, T., Hanski, I.K., 2018. The prevalence and correlates of anticoagulant rodenticide exposure in non-target predators and scavengers in Finland. Sci. Total Environ. 642, 701–707. https://doi.org/10.1016/j.scitotenv.2018.06.063.

Lambert, M.R.K., 1997. Effects of Pesticides on Amphibians and Reptiles in Sub-Saharan Africa. In Reviews of Environmental Contamination and Toxicology, vol. 150, Springer New York, p. 31–73. https://doi.org/10.1007/978-1-4612-2278-1_2.

Lambert, O., Pouliquen, H., Larhantec, M., Thorin, C., L'Hostis, M., 2007. Exposure of Raptors and Waterbirds to Anticoagulant Rodenticides (Difenacoum, Bromadiolone, Coumatetralyl, Coumafen, Brodifacoum): Epidemiological Survey in Loire Atlantique (France). Bull. Environ. Contam. Toxicol. 79, 91–94. https://doi.org/10.1007/s00128-007-9134-6.

Langford, K., Reid, M., Thomas, K., 2013. The occurrence of second generation anticoagulant rodenticides in non-target rap-tor species in Norway. Sci. Total Environ. 15, 450-451. https://doi.org/10.1016/j.scitotenv.2013.01.100.

Lemus, J.A., Bravo, C., García-Montijano, M., Palacín, C., Ponce, C., Magaña, M., 2011. Side effects of rodent control on non-target species: Rodenticides increase parasite and pathogen burden in great bustards. Sci. Total Environ. 409, 4729–34. https://doi.org/10.1016/j.scitotenv.2011.07.007.

Lohr, M.T., 2018. Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. Sci. Total Environ. 643, 134–144. https://doi.org/10.1016/j.scitotenv.2018.06.207.

López, R.M., Múrcia, D.B., 2008. First description of malignant retrobulbar and intracranial teratoma in a lesser kestrel (Falco naumanni). Avian Pathol. 37, 413–414. https://doi.org/10.1080/03079450802216660.

López-Perea, J.J., Camarero, P.R., Sánchez-Barbudo, I.S., 2019. Mateo R. Urbanization and cattle density are determinants in the exposure to anticoagulant rodenticides of non-target wildlife. Environ. Pollut. 244, 801–808. https://doi.org/10.1016/j.envpol.2018.10.101.

López-Perea, J.J., Mateo, R., 2018. Secondary Exposure to Anticoagulant Rodenticides and Effects on Predators. In Anticoagulant Rodenticides and Wildlife Vol 5, Springer International Publishing;USA, pp. 159–193. https://doi.org/10.1007/978-3-319-64377-9_7.

Marques, J., 2017. Pesquisa de rodenticidas em corujas-das-torres (*Tyto alba*).Thesis, Master of Veterinatry Medicine, Universidade Lusófona de Humanidades e Tecnologias Faculdade de Medicina Veterinária, 2017.

Martínez-Padilla, J., López-Idiáquez, D., López-Perea, J.J., Mateo, R., Paz, A., Viñuela, J., 2017. A negative association between bromadiolone exposure and nestling body condition in common kestrels: management implications for vole outbreaks. Pest Manag. Sci. 73, 364–370. https://doi.org/10.1002/ps.4435.~

Murray, M.H., Sánchez, C.A., 2021. Urban rat exposure to anticoagulant rodenticides and zoonotic infection risk. Biol Lett. 17, 20210311. https://doi.org/10.1098/rsbl.2021.0311.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. J Vet Med Si. 81, 298–313. https://doi.org/10.1292/jvms.17-0717.

Newton, I., Wyllie, I., Freestone, P., 1990. Rodenticides in British Barn Owls. Environ. Pollut. 68, 101–117. https://doi.org/10.1016/0269-7491(90)90015-5.

Newton, I., Wyllie, I., Gray, A., Eadsforth, C.V., 1994. The toxicity of the rodenticide flocoumafen to barn owls and its elimination via pellets. Pestic. Sci. 41, 187–193. https://doi.org/10.1002/ps.2780410304.

Oliva-Vidal, P., Martínez, J.M., Sánchez-Barbudo, I.S., Camarero, P.R., Colomer, M.À., Margalida, A., 2022. Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers. Environ. Pollut. 315, 120385. https://doi.org/10.1016/j.envpol.2022.120385.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BM. 372, n71. https://doi.org/10.1136/bmj.n71.

Peetris, P., 2019 Second generation anticoagulant rodenticides in birds of prey of Estonia. Thesis. Master. Eesti Maaüliko.

Ruiz-Suárez, N., Boada, L.D., Henríquez-Hernández, L.A., González-Moreo, F., Suárez-Pérez, A., Camacho, M. 2015. Continued implication of the banned pesticides carbofuran and aldicarb in the poisoning of domestic and wild animals of the Canary Islands (Spain). Sci. Total Environ. 505, 1093–1099. https://doi.org/10.1016/j.scitotenv.2014.10.093.

Salim, M.N.H, Hamid, N.H., Omar, D., KASIM, A., Zainal Abidin, C.M.R, 2014. Secondary poisoning of captive barn owls *Tyto alba* javanica through feeding with rats poisoned with chlorophacinone and bromadiolone. J. Oil Palm Res. 26, 62–72.

Sánchez-Barbudo, I.S., Camarero, P.R., Mateo, R., 2012. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Sci. Total Environ. 420, 280–288. https://doi.org/10.1016/j.scitotenv.2012.01.028.

Shawyer C., 1989. The Barn Owl in the British Isles: its past, present and future. 1st Ed. Abe-Books, Lichfield, United Kingdom.

Shore, R.F., Walker, L.A., Potter, E.D., Chaplow, J.S., Pereira, M.G., Sleep, D., 2018. Second generation anticoagulant rodenticide residues in barn owls 2018. CEH contract report to the Campaign for Responsible Rodenticide Use (CRRU) UK. pp 22.

Stone, W.B., Okoniewski, J.C., Stedelin, J.R., 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. J. Wildl. Dis. 35, 187–193. https://doi.org/10.7589/0090-3558-35.2.187.

Thomas, P.J., Mineau, P., Shore, R.F., Champoux, L., Martin, P.A., Wilson, L.K.,2011. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. Environ. Int. 37, 914–920. https://doi.org/10.1016/j.envint.2011.03.010.

Valverde, I., Espín, S., Gómez-Ramírez, P., Navas, I., María-Mojica, P., Sánchez-Virosta, P., 2021. Wildlife poisoning: a nov-el scoring system and review of analytical methods for anticoagulant rodenticide determination. Ecotoxicology. 30, 767–782. https://doi.org/10.1007/s10646-021-02411-8.

Walker, L.A., Llewellyn, N.R., Pereira, M.G., Potter, E.D., Molenaar, F.M., 2010. Anticoagulant rodenticides in predatory birds 2007 & 2008: a Predatory Bird Monitoring Scheme (PBMS) report 2010. http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_2007-8.pdf (accessed 29 November 2022).

Walker, L.A., Turk, A., Long, S.M., Wienburg, C.L., Best, J., Shore, R.F., 2008. Second generation anticoagulant rodenticides in tawny owls (*Strix aluco*) from Great Britain. Sci. Total Environ. 392, 93–98. https://doi.org/10.1016/j.scitotenv.2007.10.061.

Watt, B.E., Proudfoot, A.T., Bradberry, S.M., Vale, J.A., 2005. Anticoagulant rodenticides. Toxicol. Rev. 24, 259–269. https://doi.org/10.2165/00139709-200524040-00005.