Anticoagulant Rodenticide Exposure in Six Nocturnal Raptor Species from Madrid (Spain).

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ABSTRACT

Anticoagulant rodenticides (ARs) have been used for rodent control in both agricultural and urban settings, serving as biocides and crop protection products. Among them, second-generation anticoagulant rodenticides (SGARs) are particularly concerning due to their high persistence and bioaccumulative potential. These compounds can spread through the food chain, affecting various non-target species, with nocturnal raptors especially vulnerable as apex predators.

These substances act by inhibiting the enzyme vitamin K epoxide reductase, which prevents the activation of blood clotting factors, causing internal and external bleeding and, ultimately, the death of the animal. Even sublethal doses can lead to clinical symptoms such as anorexia, lethargy, and weakness, increasing the risk of trauma or secondary mortality.

This study aimed to assess the prevalence of AR exposure in six nocturnal raptor species in the Community of Madrid: Barn Owl (*Tyto alba),* Tawny Owl (*Strix aluco*), Eurasian Eagle-Owl (*Bubo bubo*), Long-eared Owl (*Asio otus*), Little Owl (*Athene noctua*), and Eurasian Scops Owl (*Otus scops*). A total of 164 individuals admitted to the GREFA wildlife rehabilitation center between 2017 and 2024 were analyzed. Liver samples were examined as the target biological matrix after a systematic necropsy and were tested at the Toxicology Service (SERTOX) of the University of Las Palmas de Gran Canaria using UHPLC-MS/MS.

Rodenticides were detected in 71.95% of the birds, predominantly SGARs, with bromadiolone being the most common. Additionally, 64.41% of the positive cases had more than one rodenticide detected, with the most frequent combination being brodifacoum and bromadiolone. Most detected concentrations were ≤100 ng/g, although some exceeded established toxicity thresholds.

Statistical analyses showed that adult birds had significantly higher exposure. Species feeding mainly on small mammals had higher residue levels, although insectivorous species also exhibited notable exposure. These results are consistent with previous studies from mainland Spain in diurnal raptors; however, as expected, are lower than those reported in the Canary Islands. This study underscores the need for more ecologically sustainable rodent control strategies to minimize the impact on non-target wildlife.

1. INTRODUCTION

1.1. Anticoagulant Rodenticides

1.1.1 History and Classification

By 1940, Karl Paul, in his research to determine the cause of “the bleeding disease” in cattle after ingesting sweet clover, isolated a component similar in structure to vitamin K, “dicumarol”, leading to the development of warfarin and the so-called first-generation anticoagulant rodenticides (hereafter, FGARs). Registered as a rodenticide in the 1950s, warfarin required repeated consumption to be lethal but remained highly effective for three decades. However, FGAR-resistant rodents emerged, leading to the development of more potent and persistent compounds, known as second generation anticoagulant rodenticides (hereafter, SGARs), which require only a single dose to be effective (Nakayama et al., 2019).

SGARs were developed to make them more resistant to hepatic metabolism by modifying the 4-hydroxycoumarin molecule (Table_1). The goal was to reduce the LD_{50} of FGARs and increase their $T_{1/2}$, thereby enhancing their bioaccumulation potential and indirectly increasing the risk of secondary poisoning in higher trophic levels of wildlife (Carrera et al., 2024; Cooke et al., 2023). Nevertheless, SGAR- resistant rodents have already been reported in Europe and worldwide, primarily due to mutations in the VKORC1 gene*,* which alter the function of the VKOR enzyme and reduce the efficacy of anticoagulant rodenticides (Ishizuka et al., 2008).

Table 1. Anticoagulant rodenticide classification (Nakayama et al, 2019).

Derivative	FGARs	SGARs
Hydroxycoumarin	Coumachlor, Coumatetralyl Warfarin	Difenacoum, Difethialone, Brodifacoum Bromadiolone, Flocoumafen
Indandione	Clorophacinone Diphacinone	-

1.1.2 Mechanism of Action

After ingestion, anticoagulant rodenticides inhibit the enzyme vitamin K epoxide reductase (VKORC), which is responsible for activating clotting factors II, VII, IX, and X, as well as the anticoagulant proteins C and S. Consequently, the conversion of prothrombin to thrombin is impaired, leading to internal and external bleeding (Nakayama et al., 2019). In birds, the coagulation process is more vitamin K-dependent, and their baseline levels are lower than those in mammals, making them more vulnerable (Horak et al., 2018).

The substance is absorbed in the intestine, enters the bloodstream, and is transported to the liver, where it accumulates. The liver acts as a storage site for rodenticides, making it the preferred matrix for toxicological analysis specially for SGARs, which are more lipophilic (Ishizuka et al., 2008). However, in birds, part of the ingested compound flowed directly to the kidneys via coccygeal-mesenteric vein, allowing some rodenticides to be excreted renally (Horak et al., 2018).

Metabolism occurs in the liver via the cytochrome P450 enzyme system, which transforms these compounds into hydroxylated metabolites for excretion (Ishizuka et al., 2008). This metabolic process varies by species; for example, owls have a reduced ability to metabolize warfarin due to lower CYP450 activity. As a result, rodenticide biotransformation is slower, leading to prolonged retention in the body and greater tissue accumulation, thereby increasing susceptibility (Horak et al., 2018).

Finally, excretion occurs via feces or urine and depends on the specific compound. Elimination times vary significantly, from over 100 days for flocoumafen to 27–34 hours for warfarin in chickens. Overall, enterohepatic recirculation and the high liver affinity of SGARs enhance their retention and promote fecal excretion. In contrast, renal excretion is more common in FGARs due to their hydrosolubility (Horak et al., 2018).

1.1.3 Clinical Signs, Diagnosis and Treatment

Clinical signs may appear 2-5 days after ingestion, corresponding to the time required for the depletion of functional clotting factors (Horak et al., 2018). This leads to impaired coagulation, anemia and spontaneous hemorrhages. Hemorrhagic lesions may occur in the glottis, trachea, oral cavity, keel, abdominal cavity, liver, and kidneys. The symptoms more commonly observed in recovery centers are lethargy, depression, inactivity, and pale mucous membranes, which are likely unspecific. In some cases, clinical signs are not shown, and only anorexia and depression are observed (Murray, 2011).

Primary poisoning could lead to death whereas secondary ingestion, may result in sub-lethal intoxication, which, while not always directly fatal, can ultimately be related to the mortality increase in power lines or other traumatic episodes (Hindmarch & Elliott, 2018). For this reason, distinguishing between trauma and poisoning can sometimes be challenging.

In laboratory analysis, a decreased hematocrit could be observed, often accompanied by extramedullary regeneration of red blood cells. In birds, the normal value is 35-45%, but it can decrease to as low as 6-9% (Murray, 2011). However, these findings depend on the dose ingested. In histopathological findings, microscopic and macroscopic hemorrhages, hepatic necrosis, and tissue hypoxia, among others, are observed (Rattner et al., 2011).

The diagnosis in live birds is generally based on clinical signs, however coagulation tests can be done. Imaging studies, such as CT scans or ultrasounds, help identify internal bleeding too. Definitive confirmation should be done by the detection of rodenticides in blood of live birds through liquid chromatography. However, due to the limited persistence of these compounds in avian blood, false negatives may occur (Murray, 2011). In the case of dead animals, exposure confirmation using the same laboratory techniques is carried out using the liver as the sample of choice (Badry et al., 2020).

Treatment for affected birds includes 0.2-2.2 mg/kg IM Vitamin K1 4-8h. Then 24h x 14 days after stabilized (Carpenter et al., 2016). Clinically affected patients require fluid resuscitation and possible plasma or blood transfusions, with PT monitoring every 6–12 hours until stable. Intralipid emulsion therapy has been proved and has shown successful results. Supportive care, including gastrointestinal support and oxygen therapy if signs of hypoxia are shown are needed, as well as activity restriction until coagulation normalizes (Schmidt et al., 2023).

1.1.4 Exposure Routes in Wildlife.

Regarding the formulation of ARs, they are manufactured to be palatable to rodents, with oral ingestion being the primary route of exposure. Different formats can be found, such as blocks, pastes and pellets, and they are usually colored blue or green.

In target species, exposure occurs directly through bait ingestion. However, other small non-target species such as mammals, reptiles or invertebrates' access to the baits due to their small size (Nakayama et al., 2019). Likewise, in cases of intentional poisoning of wildlife, there is also direct consumption of bait strategically prepared for the specific species.

Nevertheless, the most studied exposure route in wildlife is secondary or indirect exposure, which occurs after the ingestion of a previous intoxicated prey. Birds of prey, especially those that feed on rodents, are highly susceptible to this form of exposure (Badry et al., 2020). In general, as top predators in the food chain, they are particularly vulnerable to ecosystem disturbances Additionally, tertiary contamination along the food chain has also been reported after the ingestion of birds or other animals previously fed on an intoxicated prey (López-Perea & Mateo, 2018).

The presence of these compounds in the previously mentioned groups, as well as in amphibians, fish, crabs and marine biota confirms their bioaccumulative capacity and integration into different levels of the food chain (López-Perea & Mateo, 2018).

1.1.5 Impact on Raptors and Current Regulation

1.1.5.1. Impact on Raptors

Birds of prey survival are increasingly threatened by human activities that alter their habitats and put them at risk. One of the most significant dangers that these birds face is habitat destruction and transformation. Another major threat comes from road traffic or power lines (large species, such as Barn owl, are especially vulnerable). Furthermore, hunting remains also a problem, as well as poisoning, being a significant positive association between collision/electrocution/trauma and higher levels of toxic substances in their organism (Berny et al., 2015).

Focusing on raptor exposure to environmental contaminants, a wide body of literature has documented their involvement in poisoning incidents caused by various toxic compounds (Buij et al., 2025). Moreover, due to their ecological and biological traits, raptors have been widely used as bioindicators of environmental pollutants. As long-lived apex predators, they effectively integrate exposure over time and across broad geographic areas (Cooke et al., 2023). As part of the European Raptor Biomonitoring Facility, they have been utilized by many authors to monitor pollutants such as airborne microplastics and artificial fibers, heavy metals, PCBs, DDTs, metalloids, and polycyclic aromatic hydrocarbons (Badry et al., 2020).

Particularly, some nocturnal raptor species exhibit key traits that make them suitable bioindicators for monitoring anticoagulant rodenticide exposure (Badry et al., 2020). In Spain, numerous studies have been conducted related to the incidence of rodenticides in birds. It was estimated that 53% of the birds studied had residues in their liver, with raptors being the most affected group of birds. The compounds with the highest concentrations were brodifacoum, bromadiolone, and difenacoum (Nakayama et al., 2019). Additionally, there is a higher incidence in those nonmigratory, which live nearby agricultural or urban areas, with even a greater incidence in urbanistic areas and cattle farms, being these products primarily used as biocides (Hindmarch & Elliott, 2018; López-Perea et al., 2019) .

1.1.5.2. Current Regulation

The regulation of ARs in the European Union (EU) is divided based on their use as a plant protection product in agriculture and as a biocide for pest control in urban, industrial, and public health settings.

The status of anticoagulant rodenticides as plant protection products is governed by Regulation (EC) No 1107/2009 issued by the European Parliament and the Council of the European Union (2009), which classifies them as not approved, as can be verified in the EU Pesticide database (European Comission, 2024a). However, it allows Member States to authorize their use in special circumstances, as was the case with bromadiolone in Castilla y León between 2016-2017 during a common vole infestation.

Regarding their role as biocides, in Europe, seven AR compounds are currently registered in Europe: coumatetralyl, chlorophacinone, brodifacoum, bromadiolone, difenacoum, flocoumafen, and difethialone. These compounds are mainly regulated by Regulation (EC) No. 528/2012 and Regulation (EC) No. 2016/1179 issued by the European Parliament and the Council of the European Union (2012) and the European Comission (2016), which defined the classification, labelling and packaging.

Under Regulation (EU) No. 528/2012, all products must be authorized, and the active substances must be pre-approved by the European Chemicals Agency (ECHA, 2024.). Since

2018, their availability to the public depends on the concentration of the active ingredient contained. Products with higher concentrations (30 ppm or above) are classified as reprotoxic and restricted to specialized professionals' use, requiring official certification for handling highly toxic substances (CMRs).

Finally, the Biocidal Products Directive (BPD) identified ARs as candidates for substitution due to their high toxicity and persistence. However, under the Commission Implementing Decision (EU) 2024/734 of 27th February 2024, the expiration date of Bromadiolone, Brodifacoum, Chlorophacinone, Coumatetralyl, Difenacoum, Difethialone and Flocoumafen has been postpone to 31st December 2026 (European Commission, 2024b).

1.2. Distribution, Diet and Status of the Nocturnal Raptors studied

The six bird species studied are nocturnal raptors belonging to the Order Strigiformes. The Barn Owl (*Tyto alba*) belongs to the family Tytonidae, while the other five species—the Eurasian Eagle-Owl (*Bubo bubo*), the Long-eared Owl (*Asio otus*), the Little Owl (*Athene noctua*), the Eurasian Scops Owl (*Otus scops*), and the Tawny Owl (*Strix aluco*)—belong to the family Strigidae. These species represent five of the seven nocturnal raptors described in Spain (Salgado et al., 2022c).

All of them are nocturnal birds of prey whose morphological and anatomical characteristics allow them to precisely locate their prey. Their diet consists mainly of small rodents, mammals, lizards, and insects. As shows in (Figure_1) species such as the Barn Owl, Eagle Owl, Long-ear Owl, and Tawny Owl rely mainly on small mammals as their primary food source, followed by small birds and insects (Escala Urdapilleta et al., 2009; Gamero & De Miguel, 2017; Vassilis & Haralambos, 2003). In contrast, the Scops Owl is predominantly insectivorous (Latková et al., 2012). As for the Little Owl, its diet is quite varied, with significant differences in the proportion of invertebrates and insects consumed depending on geographic location and the specific study referenced (Latková et al., 2012; Vassilis & Haralambos, 2003). Food availability influences their reproduction, with typically one clutch per year (Salgado et al., 2022a).

All the studied species are classified as LC (Least Concern) on the IUCN Red List of Threatened Species. However, populations of the Barn Owl, Eurasian Scops Owl, and Little Owl have declined significantly. In the Community of Madrid, the distribution area has decreased by 75%, with an estimated 25–37 breeding pairs of Barn Owls in 2018 (Salgado et al., 2022b). This decline is linked to the loss of nesting sites such as barns, buildings, and churches.

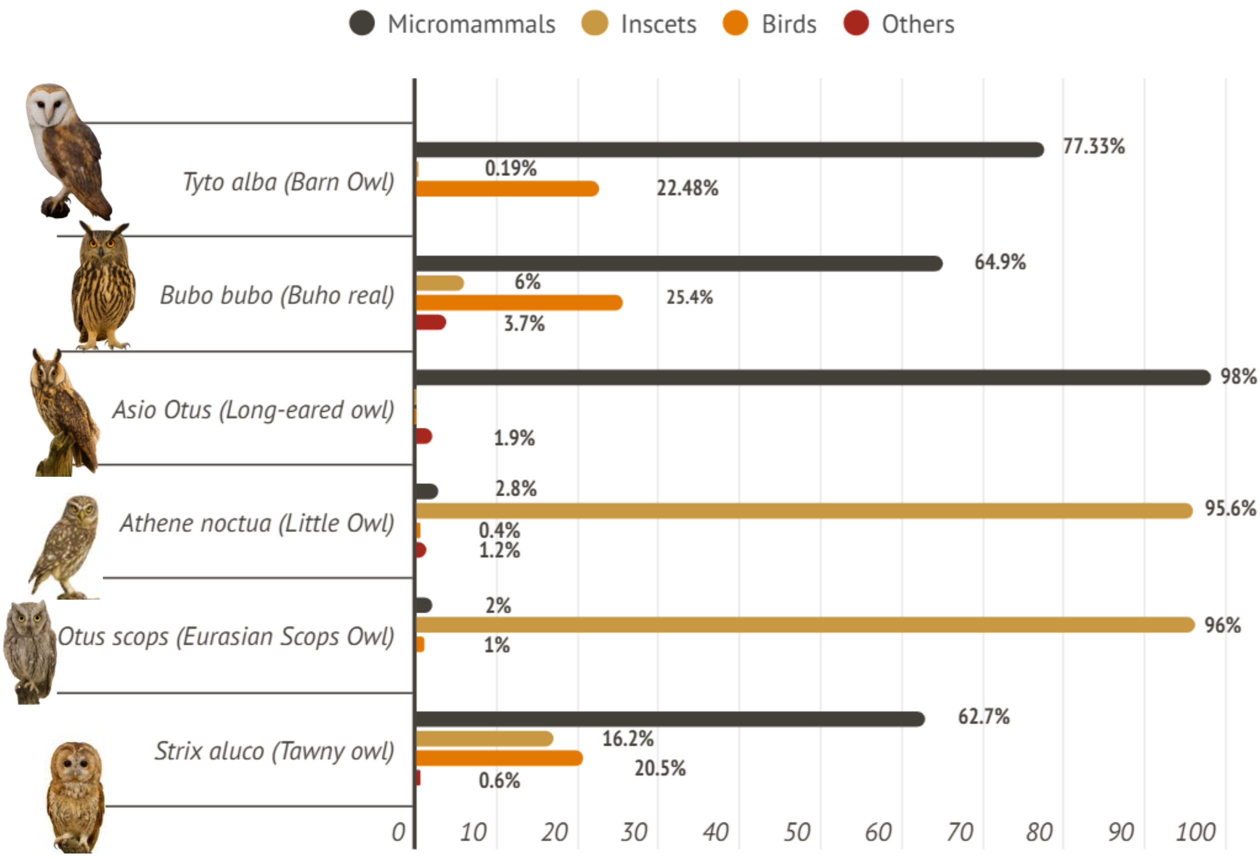

Figure 1. Graphic representation of the diet in *Tyto alba* (Garcerán, 2015), *Bubo bubo* (Fasciolo et al., 2016), *Asio* otus (Escala Urdapilleta et al., 2009), *Athene Noctua* (Vassilis & Haralambos, 2003), *Otus scops* (Latková et al., 2012), *Strix aluco* (Gamero & De Miguel, 2017).

Additionally, between 2006 and 2018, the populations of the Eurasian Scops Owl and the Little Owl declined by 32% and 24%, respectively (Fernández-Calvo, 2022; Salgado, et al., 2022c). The Scops Owl now meets the criteria to be classified as a vulnerable species in Spain (Escandell, 2019).

In contrast, despite some fluctuations, the population of the Tawny Owl, Long-eared Owl, and Eurasian Eagle-Owl have remained stable or even slightly increased in the latter two species. During winter, the Little Owl, Long-eared Owl, Barn Owl, and Eurasian Scops Owl—though sedentary in the Iberian Peninsula during the breeding season—move to their nesting areas and migrate to the south or warmer regions in winter. Meanwhile, the Tawny Owl and Eurasian Eagle-Owl are resident species with minimal movements (Escandell, 2019; Salgado et al., 2022c)

All these raptors have a broad distribution across the Iberian Peninsula, highlighting the wide distribution around the Community of Madrid. In contrast, only the Barn Owl and the Long-eared Owl are found in the Canary Islands (Salgado et al., 2022a)

2. OBJECTIVES

2.1. General Objective

The main objective of this study was to determine the exposure to anticoagulant rodenticides in the Barn Owl (*Tyto alba*), the Eurasian Eagle-Owl (*Bubo bubo*), the Long-eared Owl (*Asio otus*), the Little Owl (*Athene noctua*), the Eurasian Scops Owl (*Otus scops*), and the Tawny Owl (*Strix aluco*) in the Community of Madrid, based on nocturnal raptors admitted to the recovery and rehabilitation center, GREFA.

2.2. Specific Objectives

To achieve this goal, the following specific objectives were established:

1. Analyze and measure the concentration of anticoagulant rodenticides, both FGARs (warfarin, coumatetralyl, coumachlor and clorophacinone) and SGARs (Brodifacoum, bromadiolone, difenacoum, difetialone and flocoumafen) using the liver as a biological matrix.
2. Evaluate toxicity levels based on measured concentrations and established toxicological thresholds.
3. Examine exposure patterns in relation to the recorded biological variables.

3. MATERIAL AND METHODS

3.1 Localization and Sample Collection

The samples collected in this study were received between January 2017 and December 2024 at GREFA (The Native Fauna and Habitat Rehabilitation Centre), which is a non-profit and non-governmental organization founded in 1981, as an association for the study and conservation of nature, located in Majadahonda, Madrid.

The Community of Madrid (Figure_2) is the capital of Spain, which is situated in the center of the country with an extension of 802,800 h and a population of 7.001.715 habitants (INE, 2024), divided into 179 municipalities (Instituto Estadística, 2025). The climatology is characterized by mainly continental Mediterranean weather by cold winter and hot summers; temperatures fluctuate from minus zero to over 35 Cº respectively. The average annual rainfall is about 400 to 600mm (AEMET, 2024).The surface area used for agricultural purposes is around 28% of the national territory, whereas livestock farming represents around 16%, and the forestal area represents 54% over the total surface (MAPA, 2022).The Community of Madrid has experienced a decrease in the number of crops, whereas the area has slightly increased, what indicates a tendency towards more extensive exploitation (IDEM, 2024).

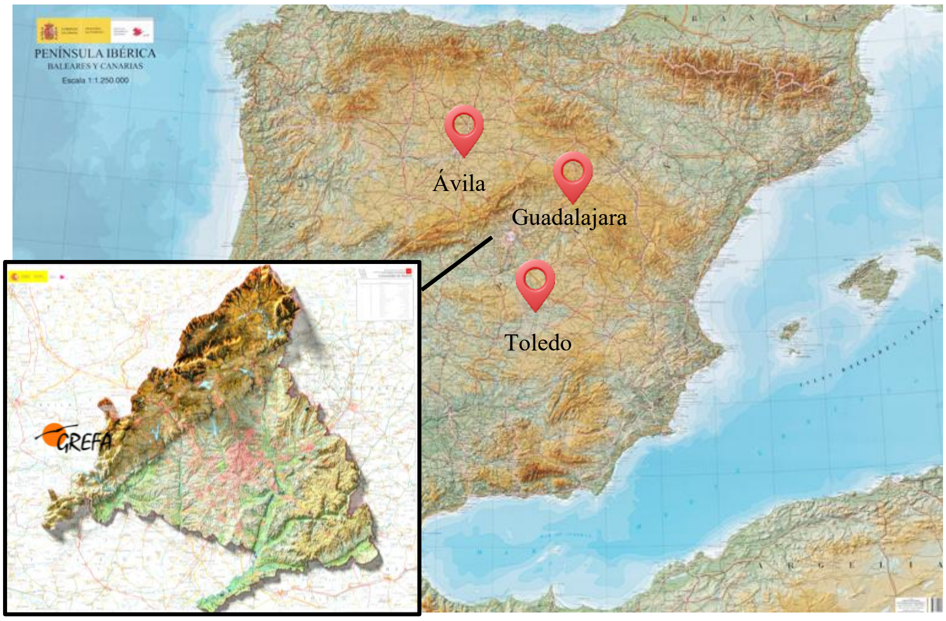

Figure 2. Map from Spain, indicating the places where the birds were collected (Ávila, Guadalajara, Madrid and Toledo).

Regarding the studied population, 164 nocturnal birds were analyzed: 90 Eagle owls (*Bubo bubo)*, 3 Barn Owls (*Tyto alba)*, 22 Tawny Owl (*Strix aluco)*, 11 Long-eared Owls (*Asio otus)*,19 Little owls (*Athene Noctua)* and 19 Eurasian Scops Owls *(Otus scops)* from the Community of Madrid, with only 2 animals from Guadalajara, 1 from Toledo and 1 from Ávila. However, the exact location was not obtained.

The data were collected using GREFA´s own clinical history management software, called "BUHO". The recorded variables included the year, province where each specimen was found, cause of admission, age, sex, and body condition at the time of the first examination.

Although necropsies were performed after death during hospitalization or euthanasia due to the severity of injuries, the exact cause of death could not be clearly determined in most cases. Therefore, the cause of admission was classified based on the information provided by the person who found the animal, the physical examination performed by the veterinarian team, or the post-mortem findings. The determination of the body condition was indicated by the size and thickness of the pectoral muscle at the admission time. The sex was obtained by the observation of the gonads, however in some cases could not be identified. The age was collected following EURING classification (Feu et al., 2020), however birds under 1 year were grouped together, so it was identified 3 ages, namely age 1: chick or unable to fly freely (n=43); age 2 juvenile between 1 and 2 years (n = 19) and adult > 2years (n = 102) (Table_2).

Table 2. Description of the variables collected (sex, age, admission cause and body condition.

Variables	Description	
Sex	Male	Male gonads observed
	Female	Female gonads observed
	Undetermined	Not identified
Age (Feu et al., 2020)	Nestling/Fledgling/ Juvenile	Unable to fly and/or under 1 year old
	Inmature	< 1 year old and under < 2 years
	Adult	>2 years
Admission cause	Trauma	Compatible to traumatism
	Nestling	Premature nest abandonment
	Natural disease	Infectious, fungal or parasite diseases
	Intoxication	Compatible to intoxication (Pb, rodenticides, insecticides…)
	Others	Not included in other groups
Body condition (Burton et al., 2013)	Cachexia	Important loss of pectoral muscle, keel very palpable
	Emaciated	Low pectoral muscle mass, palpable keel
	Thin	Mild pectoral muscle loss, slightly palpable keel
	Normal	Adequate pectoral muscle, no keel palpation
	Fat	Pectoral muscle exceeds the keel

The rodenticide analysis was carried out by sampling the liver after performing a regulated necropsy (Linares, 2013). The samples were stored at -20 Cº and then were sent to Toxicology Service of the University of Las Palmas de Gran Canaria (SERTOX).

3.2. Chemical Analysis and Sample Preparation

The method used for liver extraction was QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), which allows the analysis of different compounds in complex matrices such as food, blood, or soil and was previously validated for liver (Rial-Berriel, et al., 2021b). The procedure consisted of homogenizing 1 g of liver tissue with 4 ml miliQ water using a Precellys Evolution homogenizer from Bertin Technologies in Rockville, Maryland, USA at 6500 rpm for 2 sets of 30 second. Subsequently, 1 g of the homogenate was manually shaken with 2 mL of ACN 0.5% FA in a 5 mL Eppendorf tube and sonicated for 20 min using equipment from VWR (Selecta, Barcelona, Spain). Then, 480 mg of anhydrous magnesium sulfate and 120 mg of sodium acetate were added to each sample tube, followed by vortex mixing for 30 s and manual shaking for 1 min. After centrifugation at 4200 rpm g for 5 min at 2 °C, the supernatant was filtered through a 0.2 µm Chromafil PET-20/15 filter and finally, a clean-up phase was performed collecting 200 µl of the supernatant into glass amber vials.

For the analysis, certified procedural-internal standards (P-IS, (±)- Warfarin-d5) and certified ARs standards with the maximum purity range between 98% to 99.8%% from Dr. Ehrenstorfer in Augsburg, Germany, were added. Among the ARs included in the panel, five of them were FGARs (warfarin, chlorophacinone, coumachlor, coumatetralyl and diphacinone) and five were SGARs (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen).

The solvents used were used acetonitrile (ACN), methanol (MeOH) and formic acid (FA) with 98% purity, from Honeywell in Morristown, NJ, USA. Water for the study was produced through a MilliQ A10 water purification system by Millipore in Molsheim, France. The QuEChERS Extract Pouch, AOAC Method, contained 6 g of magnesium sulfate and 1.5 g of sodium acetate, was obtained from Agilent Technologies in Palo Alto, CA, USA.

To ensure analytical accuracy, quality control (QC) samples were included for every batch of 30 samples. A ten-point calibration curve was generated using negative chicken liver, covering concentrations from 0.195 ng/g to 100 ng/g, following the same extraction protocol. QCs were prepared at a concentration of 5 ng/g. All samples, QCs, calibration standards, and blanks were spiked with the P-IS solution prior to extraction. Results were expressed on a wet weight basis.

Finally, for the detection and quantification of ARs, an Agilent 1290 UHPLC system coupled with an Agilent 6460 triple quadrupole mass spectrometer was employed.

3.3. Statistical Analysis

Final quantification of analytes in each sample was conducted using the MassHunter Quantitative Analysis software.

The statistical analysis was made centered on SGARs as they were present in most samples, except for a single case in which coumatetralyl (a FGAR) was detected. Individuals with concentrations above the limit of quantification (LOQ) (Table_3) or between the limit of detection (LOD) and the LOQ were considered positive. For statistical purposes, values between LOD and LOQ were assigned random values within that range. Concentrations below the LOD were considered non-detects and were assigned random values between 0 and half the LOD.

Table 3. Limit of quantification (LOQ) and limit of detection (LOD) values for anticoagulant rodenticides (Rial-Berriel et al., 2021a).

GROUP	Rodenticide	LOQ	LOD
FGARs	Coumatretralyl	1.6	1.36
	Clorophacinone	8	6.8
	Diphacinone	8	6.8
	Coumachlor	0.8	0.68
	Warfarin	0.8	0.68
SGARs	Brodifacoum	0.4	0.34
	Bromadiolone	0.4	0.34
	Difenacoum	0.8	0.68
	Difethialone	1.6	1.36

Flocoumafen	0.4	0.34

Descriptive statistics included detection frequency (percentage of birds with at least one rodenticide detected), median, mean, interquartile range (25th and 75th percentiles), and standard deviation. The minimum and maximum concentration values for each detected rodenticide were also reported.

Rodenticide concentrations were categorized into four ranges (<50 ng/g, 50–100 ng/g, 100–200 ng/g, and >200 ng/g), based on thresholds considered relevant for toxicity interpretation (Lohr, 2018).

For comparative analyses, non-parametric tests were used due to the non-normal distribution of the dataset. The Kruskal–Wallis test was applied to assess differences across age groups, body condition, and species, while the Mann–Whitney U was used to compare differences between sexes. Post hoc pairwise comparisons were made to determine where the statistically significant differences lay among the groups. A p-value ≤ 0.05 was considered statistically significant.

All statistical analyses were performed using Jamovi and Microsoft Excel, while data visualizations were created using Microsoft Office, Infogram, and Canva.

4. RESULTS

4.1. Characteristics of the Dataset

The studied population (n=164) was composed by 90 Eagle Owls, (*Bubo bubo*), 3 Barn Owls (*Tyto alba*), 22 Tawny Owls (*Strix aluco*), 11-Long-eared Owls (*Asio otus),* 19 Little Owls (*Athene Noctua*) and 19 Eurasian Scops Owls (*Otus scops*). The sample collection period was from 2017 to 2024, with the highest representation in 2019 (n=37) accounting for 22.5% of the total samples.

Concerning the characteristics of the population (Figure_3), the proportion of males was 34% (n=53), 53% were female (n=87) and 14,6% was not determined (n=24). Divided by species, the Long-eared Owl had the highest proportion of males (63.63%) and for the Barn Owl, all individuals were females. In case of the Scops Owl, it showed the greatest difficulty in identifying the sex.

Regarding the age, in most species, the predominant age group was adulthood, except for Little owls *(Athene noctua)* and Scops owls *(Otus scops),* in which chicks or unable to fly showed the highest prevalence. In addition, related to body condition, the normal condition was the most abundant with 43.2%, following by thin birds in 25% of the cases.

Finally, the primary cause of admission was trauma (66.46%), followed by nestling admission (15.24%) and natural diseases (9.76%). In the last place it was poisoning (0.61%), which includes pesticides, Pb or rodenticides intoxication.

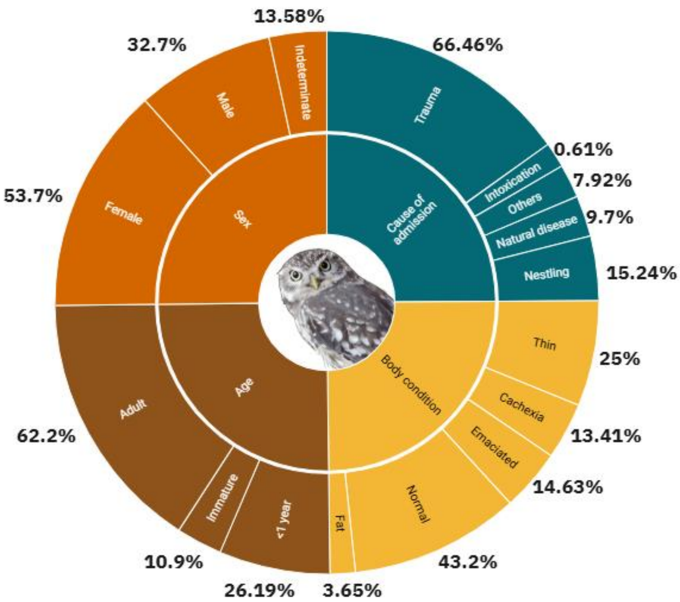

Figure 3. Characteristics of the studied population (n=164), accordingly to the variables sex, cause of admission, body condition and age.

4.2. Descriptive Analysis of ARs found in Nocturnal Raptors from Madrid

Out of the 164 analyzed samples, 71.95% (n=118) showed detectable concentrations of anticoagulant rodenticides. Among the positive cases, all were identified as SGARs (Second-Generation Anticoagulant Rodenticides), except for one isolated case of the FGAR coumatetralyl, which was detected in a *Strix aluco* specimen with 5.46 ng/g. The most frequently detected rodenticide was bromadiolone, present in 76.27% (n=90) of the positive samples, being the most frequent compund in all species except for *Athene noctua*, in wich brodifacoum was the predominant. Following bormadiolone, the most frequent compounds were brodifacoum 72.8% (n=86) , difenacoum 34.74% (n=41), difethialone 14.4%, (n=17), and flocoumafen 13.55% (n=16) *(*Figure 4).

Among all positive cases, a single SGAR compound was detected in 42 instances (35.59%), most commonly bromadiolone. Two compounds were identified together in 36 cases (30.51%), with the most frequent being bromadiolone and brodifacoum. Three compounds were detected simultaneously in 28 cases (23.73%), often involving difenacoum. Four compounds were identified together in 7 cases (5.93%), tipically with flocoumafen added to the previous combination. Lastly, five compounds were detected in 5 cases (4.2%), including difethialone in the mix. In all cases, where all five SGARs were simultaneously present, the affected species was the Eurasian eagle-owl *(Bubo bubo) (*Figure 4).

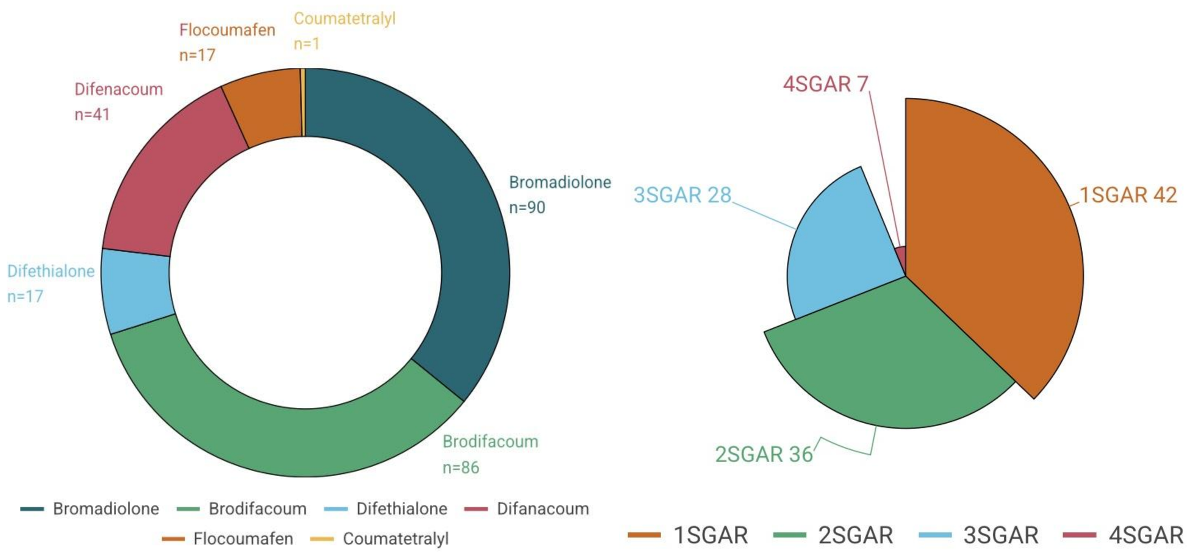

Figure 4. Distribution of detected anticoagulant rodenticides among positive cases (n=118) ***(Left).*** Rodenticide combinations detected by groups of 1,2,3,4 or 5 rodenticides detected simultaneously ***(Right).***

Among the detected compounds (Anexo 1), the highest concentration corresponded to bromadiolone, reaching a maximum concentration of 401.33 ng/g in a *Strix aluco*. This was followed by brodifacoum with 189.32 ng/g, found in an *Athene noctua*. The maximum concentrations recorded for the remaining SGARs were 30.62 ng/g of difethialone in an *Athene noctua*, 50.88 ng/g of difenacoum in *Bubo bubo*, and 3.12 ng/g of flocoumafen in a *Tyto alba.*

4.2.1. Toxicity Threshold Categorization for SGARs

The detected concentrations (Figure_5) were categorized into four groups. Among the positive cases, 65.25% had concentrations below 50 ng/g, 19.49% showed concentrations between 50-100 ng/g, and 11.86% fell within the 100-200 ng/g range. Lastly, only 3.39% had concentrations exceeding 200 ng/g.

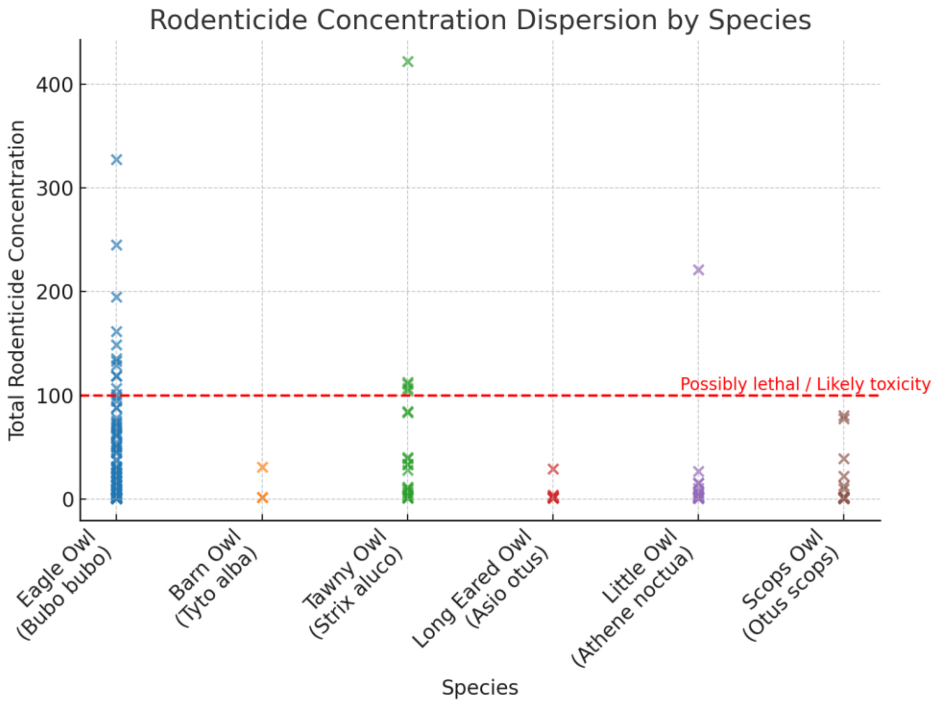

Figure 5. Rodenticide concentration dispersion by Species (*Bubo bubo, Tyto alba, Strix aluco, Asio otus, Athene noctua, Otus scops*). The discontinuous red line is situated at 100 ng/g, from which the toxicity risk is categorized as possibly lethal/ likely toxicity (Lohr, 2018).

4.2.2. Variables Variation in SGAR Exposure

Regarding species exposure to rodenticides (Table_4) *Bubo bubo* and *Strix aluco* were the most affected birds in terms of ΣSGARs, with detection frequencies of 78.8% (n = 71) and 86.4% (n =19), respectively. *Bubo bubo* exhibited widespread contamination, with detections of all analyzed compounds, including simultaneous presence of all five SGARs. Notably, this species showed a mean concentration of 56.80 ng/g and a maximum of 327.60 ng/g. Similarly, *Strix aluco* displayed the highest individual concentration recorded (422.06 ng/g), with a mean of 65.65 ng/g—higher than that of *Bubo bubo* and practically all other species—thus making it the most affected species overall.

Next, in *Athene noctua* and *Tyto alba*, rodenticides were detected in over half of the analyzed samples—approximately 68% (n = 13) and 66.6% (n = 2), respectively. Although concentrations were generally low, the mean values were 24.4 ng/g for *A. noctua* and 16.22 ng/g for *T. alba*, with a notable outlier of 220 ng/g detected in one *A. noctua* specimen. Brodifacoum and bromadiolone were detected in both species; however, *A. noctua* also exhibited traces of difenacoum and difethialone, whereas *T. alba* was characterized by the presence of flocoumafen.

Finally, the species with the lowest detection frequencies were *Otus scops* and *Asio otus*, with 47.3% (n = 9) and 36.36% (n = 4), respectively. In the case of *O. scops*, the mean concentration was 27.57 ng/g, reaching a maximum of 80.54 ng/g. *Asio otus* showed concentrations below 29 ng/g, with a mean of 9.49 ng/g, making it the species with the lowest incidence. In this case, difethialone was the compound with the highest concentration, whereas *O. scops* had bromadiolone as the primary detected compound.

Additionally, non-parametric test were performed to explore to explore significant differences among the studied species. The Kruskal–Wallis test revealed significant differences in anticoagulant rodenticide exposure between species ($p < 0.001$). Post hoc pairwise comparisons using the Dwass–Steel–Critchlow–Fligner test indicated that *Bubo bubo* (Eurasian Eagle Owl) exhibited significantly higher concentrations than *Otus scops* (Scops Owl) ($p = 0.032$) and *Strix aluco* (Tawny Owl) ($p = 0.027$), as did *Strix aluco* compared to *Otus scops* ($p = 0.040$). Additionally, *Athene noctua* (Little Owl) showed significantly lower levels than both *Bubo bubo* ($p = 0.026$) and *Strix aluco* ($p = 0.034$). No significant differences were observed between *Tyto alba* (Barn Owl) and the other species. However, these results should be interpreted with caution due to the unbalanced sample distribution among species. Future studies should aim to include larger sample sizes and more robust statistical analyses to validate these findings.

Table 4. Summary table of ∑SGARs detected in nocturnal raptors in the Community of Madrid, Spain.

Specie	Frequency	Mean [ng/g]	Median [ng/g]	Max [ng/g]	Min [ng/g]	P25-P75	SD
***Strix aluco* n=22**	86.36 % (n=19)	65.65	33.60	422.06	3.09	9.83 – 94.43	95.47
***Bubo bubo* n=90**	78.8 % (n=71)	56.80	38.66	327.60	1.73	17.47 – 74.11	59.19
***Athene noctua* n=19**	68.4 % (n=13)	24.4	5.9	220.9	1.51	2.67 – 14.82	59.49
***Tyto alba* n=3**	66.6 % (n=2)	16.22	16.22	30.94	1.51	8.87 – 23.58	20.8
***Otus scops* n=19**	47.3 % (n=9)	27.57	13.02	80.54	1.63	2.27 – 38.99	31.51
***Asio otus* n=11**	36.36 % (n=4)	9.49	3.28	29.37	2.03	2.42 – 10.35	13.28

Note: Data include frequency of detection, mean, median, maximun (max) and minimun (min) concentration detected, percentile 25-75 (P25, P75) and standard deviation (SD).

Regarding the remaining recorded variables, non-parametric tests were conducted to evaluate statistically significant differences. Among all sampled individuals, females showed a higher detection rate than males (see Table_5); however, this difference was not statistically significant ($p = 0.435$).

The most frequent body condition was classified as "normal" (43%), with 64.78% of these individuals testing positive. Nevertheless, no significant association was found when compared to individuals with low or high body condition ($p = 0.412$).

Trauma was the most common cause of admission (64.46%), and among these cases, 75.23% tested positive for rodenticides. However, due to the unequal sample size distribution among the different causes of admission, statistical comparisons between groups could not be performed.

Finally, the most frequent age group was Age 3 – Adult (62.19%), which also accounted for 82.85% of all positive cases ($n = 118$) and exhibited the highest mean rodenticide concentration (55.34 ng/g). A non-parametric test revealed a significant difference between age groups, with adults being significantly more exposed ($p < 0.001$)

Table 5. Descriptive table of rodenticide detection across categorical variables and p-values from non-parametric test analysis.

Variable	Category	n total	Not detected (n)	Not detected (%)	Detected (n)	Detected (%)	p-value
Sex	Male	53	16	30.19%	37	69.81%	0.435
	Female	87	20	22.99%	67	77.01%	
	Unsexed	24	10	35.36%	14	64.64%	
Age	Age-1	43	22	51.17%	21	48.83%	<0.001
	Age-2	16	7	43.75%	9	56.25%	
	Age-3	105	18	17.15%	87	82.85%	
Body condition	Cachexia	22	4	18.19%	18	81.81%	0.412
	Emaciated	24	5	20.84%	19	79.16%	
	Thin	41	10	24.39%	31	75.60%	
	Normal	71	26	36.62%	45	63.38%	
	Fat	6	1	16.67%	5	83.33%	
Cause of admission	Nestling	25	13	52.00%	12	48%	-
	Natural disease	16	3	18.75%	13	81.25%	
	Trauma	109	27	24.77%	82	75.23%	
	Intoxication	1	0	0.00%	1	100%	
	Others	13	3	23.08%	10	76.92%	
Total sampled		164	46	28.04%	118	71.95%	

5. DISCUSSION

Raptors are widely used as sentinels of environmental contamination across Europe. Nocturnal species possess ecological traits that make them especially suitable for monitoring anticoagulant rodenticides, with some ranked among the most effective biomonitoring species (Badry et al., 2020).

This study analyzed 164 liver samples from six nocturnal raptor species collected in the Community of Madrid and nearby provinces (Ávila, Guadalajara, and Toledo). This region includes both highly urbanized areas and more rural zones dedicated to livestock farming and agriculture—land uses that are recognized predictors of rodenticide contamination and can act as "ecotoxicological traps"(López-Perea & Mateo, 2018). However, the lack of precise location data for most samples precluded a spatial analysis of risk factors or land-use associations.

5.1 Descriptive Analysis of AR Exposure in Nocturnal Raptors

Out of the 164 liver samples analyzed, 71.95% contained detectable concentrations of at least one anticoagulant rodenticide (AR), a prevalence that aligns with global findings, where detection rates in raptors frequently range between 60% and over 90% (Christensen et al., 2012; Ruiz-Suárez et al., 2014; Sánchez-Barbudo et al., 2012; Spadetto et al., 2024, 2025). However, AR exposure varies widely depending on species, region, season, and food availability, among other factors (Christensen et al., 2012). In Spain, both nocturnal and diurnal raptors have shown high prevalence rates consistent with these global trends (Carrillo-Hidalgo et al., 2024; López-Perea et al., 2015; Rial-Berriel et al., 2021a; Sánchez-Barbudo et al., 2012). Nevertheless, lower values have also been reported in the UK (Walker et al., 2008), and in studies when blood is used as the biological matrix, given the lower persistence of these compounds in blood compared to other tissues (Spadetto et al., 2025). Additionally, some authors have reported higher detection rates in insular environments, such as the Canary and Balearic Islands, compared to mainland regions—likely linked to differences in land use, rodent control practices, possible rodent resistance and ecological dynamics (López-Perea et al., 2015; Martín Cruz et al., 2024a).

All detected compounds belonged to the group of second-generation anticoagulant rodenticides (SGARs), except for a single case involving coumatetralyl (a FGAR). This finding reflects the global trend toward a decreasing use and detection of first-generation rodenticides, attributed to factors such as increased rodent resistance, stricter regulations, and lower persistence in the environment (Carrillo-Hidalgo et al., 2024; Ishizuka et al., 2008; Martín Cruz et al., 2024a).

Bromadiolone was the most frequently detected AR in this study (76.27%), contrasting with findings from other studies where brodifacoum predominated (Carrillo-Hidalgo et al., 2024; Fourel et al., 2024; Langford et al., 2013; Martín Cruz et al., 2024a). In our case, brodifacoum was the second most frequent compound, followed by difenacoum, difethialone, and flocoumafen. This detection pattern, in which brodifacoum and bromadiolone are the most frequently found compounds, likely reflects their widespread use in Spain, where many commercial baits authorized for general public use contain these SGARs, as documented in the 'Registro Oficial de Biocidas de España' (Ministerio de Sanidad, 2025).

These compounds were frequently detected in combination, a pattern commonly observed in raptor studies worldwide (Carrillo-Hidalgo et al., 2024; Christensen et al., 2012; Cooke et al., 2023; Spadetto et al., 2024). Up to 15 different combinations of rodenticides were identified, and some individuals—such as the Eurasian eagle-owl—tested positive for as many as five ARs simultaneously. The presence of multiple SGARs in a single individual suggests repeated exposure to various bait formulations over time. This type of co-exposure may have more severe toxicological consequences than single-compound exposure, even at similar doses, due to the differing physicochemical properties and mechanisms of action of each compound. These interactions can lead to additive or even synergistic effects (Lohr, 2018).

5.1.1. Toxicity Threshold Interpretation

Regarding the concentrations detected, over half of the samples contained less than 50 ng/g. An additional 31.35% had concentrations between 50 and 200 ng/g, and 3.39% exceeded 200 ng/g—levels at which lethal effects have been previously reported (Lohr, 2018). While these thresholds are commonly used, more recent studies indicate that toxicity thresholds can vary substantially between species and compounds (Elliott et al., 2024). For example, the probability of intoxication in Barn owls reaches 50% at concentrations as low as 39 ng/g, while True owls (Strigidae) require approximately 107 ng/g to reach the same risk level. Similarly, for specific compounds, 106 ng/g of bromadiolone is needed to reach a 50% toxicity probability, whereas only half that concentration of brodifacoum or difethialone can produce equivalent effects. Based on these findings, one of the Barn Owls in our study could be at higher risk of rodenticide poisoning, as it showed a concentration close to 39 ng/g. Similarly, 11 Eurasian Eagle Owls, 4 Tawny Owls, and 1 Little Owl presented concentrations close to or exceeding 107 ng/g. Likewise, for the remaining individuals with lower concentrations, sublethal exposures may still contribute to physiological weakening and increased mortality in raptors (Murray, 2011).

The proportion of individuals exceeding toxicity thresholds in our study is lower than that reported in comparable research conducted in Catalonia and Mallorca, where 23.3% of avian predators had concentrations above 200ng/g (López-Perea et al., 2015). Similar results were found in the Canary Islands and Valencian Community, where 35% of analyzed raptors exceeded the 100–200 ng/g range (Ruiz-Suárez et al., 2014; Vicedo et al., 2024) and nearly 50% of kestrels in Tenerife had levels above this threshold (Carrillo-Hidalgo et al., 2024). In our study, the highest concentration was 422 ng/g in a Tawny owl, whereas in insular regions of Spain, concentrations above 1,000 ng/g have been reported (Carrillo-Hidalgo et al., 2024) including an extreme case of over 9,000 ng/g in a Long-eared owl (Martín-Cruz et al., 2024b), which was associated with intentional poisoning.

5.2. Analysis of Biological and Ecological Factors Related to AR Exposure

5.2.1. Species Differences and Ecological Considerations

Species-level differences were statistically significant ($p < 0.001$), highlighting considerable variation in AR exposure among the six raptor species studied. These differences likely reflect ecological and behavioral traits, particularly dietary preferences. However, due to the heterogeneous and unbalanced sample sizes among species, robust interspecific statistical comparisons were not feasible. Instead, a descriptive approach was adopted, integrating species-specific ecological knowledge.

The Eurasian eagle owl (*Bubo bubo*) and the Tawny owl (*Strix aluco*) exhibited the highest prevalence rates (78.8% and 86.4%, respectively), along with the highest residue concentrations (See Table_4). Both species are known to prey primarily on small mammals (see Figure_1) although *S. aluco* has a more varied diet that also includes birds and insects (Gamero & De Miguel, 2017). These findings are consistent with previous studies in Spain and other European countries (Langford et al., 2013) although lower prevalence rates have been reported in France and Norway (Lambert et al., 2007; Langford et al., 2013).

In contrast, the Long-eared owl (*Asio otus*) and the Eurasian scops owl (*Otus scops*) showed the lowest detection rates (36.4% and 47.3%, respectively). *A. otus* feeds almost exclusively on micromammals (99.6) (Escala Urdapilleta et al., 2009) while *O. scops* are predominantly insectivorous (96.2%) (Latková et al., 2012). Interestingly, the prevalence in *A. otus* was lower than expected, given its diet and previous data from mainland Spain and the Canary Islands, where 100% of individuals tested positive (López-Perea et al., 2019; Rial-Berriel et al., 2021a).

The little owl (*Athene noctua*) had an intermediate prevalence (68.4%). Its diet is known to be highly variable depending on region and prey availability, shifting from rodent-dominated to insect-rich compositions (Salgado, et al., 2022c; Vassilis & Haralambos, 2003).

Although diet was not directly assessed in this study due to the absence of geolocation or stomach content data, the results support previous evidence that species with a greater reliance on small mammals tend to accumulate higher levels of ARs (Ruiz-Suárez et al., 2014). Nonetheless, some studies have found no clear distinction between specialists and generalists, with generalist species sometimes showing even higher concentrations (López-Perea & Mateo, 2018).

Furthermore, raptors that prey on birds—such as *Tyto alba*, *B. bubo*, and *S. aluco*—may experience secondary exposure via avian prey already contaminated with rodenticides, potentially leading to biomagnification (Sánchez-Barbudo et al., 2012). Similarly, recent studies have highlighted the role of invertebrates as indirect vectors, as insects can feed on bait and contribute to exposure in insectivorous species (Nakayama et al., 2019; Williams et al., 2023).

Taking together, our findings reinforce the concept that ARs are not restricted to rodent-targeting predators but are distributed throughout food webs, with multiple taxa acting as secondary vectors and contributing to the environmental spread of these toxicants.

5.2.2. Age-Related Exposure Patterns

Age recorded significant differences between groups ($p < 0.001$), with adults (Age 3) showing the highest prevalence (82%). Juveniles (Age 2) and individuals under one year old (Age 1) exhibited lower—but still substantial—prevalence rates (56% and 50%, respectively).

Although younger birds showed lower exposure, detection of ARs at early life stages is concerning due to the persistence and bioaccumulative nature of these compounds. Recent studies indicate that early-life exposure may contribute to long-term health effects and reduced survival. For instance, 98.6% of eagle owl chicks in Murcia tested positive for AR residues—a pattern linked to high local rodenticide use (Spadetto et al., 2025).These trends align with findings in other regions, where adults typically accumulate higher concentrations of ARs over time (Carrillo-Hidalgo et al., 2024).

5.2.3. Other Biological and Ecological Variables

Although females showed slightly higher exposure than males (77.0% vs. 69.8%), this difference was not statistically significant. Similar patterns have been reported in kestrels in Tenerife (Carrillo-Hidalgo et al., 2024) and in scavengers elsewhere in Spain (Oliva-Vidal et al., 2022). Conversely, some studies in other raptor species have observed slightly higher AR accumulation in males, potentially due to behavioral or physiological differences (Broughton et al., 2022). Overall, sex does not appear to be a consistent predictor of exposure, though species- and region-specific variations may occur.

Interestingly, many individuals with high residue concentrations appeared to be in normal body condition, but no significant differences relative to individuals with either low or high body condition were found. These findings indicate that some exposed individuals may remain asymptomatic. Body condition is influenced by diverse ecological and physiological factors, not all of which are directly linked to toxicant burden (Abernathy et al., 2018; Martínez-Padilla et al., 2017).

A statistical comparison of exposure based on cause of admission could not be performed due to highly unbalanced sample sizes between recorded groups. Nevertheless, it is notable that 75.2% of individuals admitted due to trauma, and 81.2% of those admitted due to natural disease tested positive for ARs. These results suggest that rodenticide exposure may compromise physiological condition and increase vulnerability to other threats. Trauma, as the most common cause of admission and, in some cases natural diseases, could be secondary to rodenticide-induced weakness or coagulopathy (Ferreiro et al., 2018; Manosa & Real, 2024). Hemorrhagic lesions may be misattributed to trauma rather than AR toxicity, complicating accurate diagnosis and underestimating the contribution of rodenticides to morbidity and mortality (Elliott et al., 2024).

Future studies should include larger, more evenly distributed sample sizes and geolocation data to better assess how biological traits and anthropogenic activities influence AR exposure in nocturnal raptors in the Community of Madrid.

6. CONCLUSIONS

1. The exposure of nocturnal raptors to anticoagulant rodenticides in the Community of Madrid exceeded 71%, indicating the widespread presence of these compounds in the environment. All species were affected, although with considerable variation among them. Notably, the Scops Owl, an insectivore, stands out, highlighting the incidence of contamination through the food chain and the processes of bioaccumulation and biomagnification within the ecosystem, including insects.

2. Most of the detected rodenticides belonged to SGARs, except for a single case involving coumatetralyl. This confirms the trend toward the predominance of biocidal products most widely authorized for use in Spain. Moreover, the simultaneous presence of multiple rodenticides was notable, occurring in more than half of the positive cases. However, concentrations were generally below 50 ng/g.

3. Apparently, there is no direct relationship to the variables studied, except for age, where it was observed that "adult" individuals (>2 years) show a higher predisposition to rodenticide exposure, likely due to the bioaccumulative nature of these compounds.

4. In response to this issue, it is recommended to support initiatives such as the installation of nest boxes, in which GREFA has been actively involved. These projects have proven effective in reducing pest populations such as voles, thereby decreasing the need for toxic substances and highlighting the crucial role of raptors in biological control. Additionally, the importance of toxicological analysis in wildlife is emphasized, as it serves as a valuable bioindicator of the presence of toxic compounds in the environment.

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Annex

Anexo 1 Frequency, mean, median, maximum, minimum, P25th, P75th standard deviation of the rodenticide incidence in the six species.

Specie	Compound	Frequency	Mean [ng/g]	Median [ng/g]	Max [ng/g]	Min [ng/]	P 25	P75	Standard Deviation	Mean ± SD
Eagle Owl *Bubo bubo*	Brodifacoum	52	15.89	11.34	83.15	1.66	3.86	19.71	16.77	15.89 ± 16.77
	Bromadiolone	62	46.03	20.31	320.64	1.30	5.95	63.89	59.86	46.03 ± 59.86
	Difenacoum	35	6.88	1.36	50.88	0.55	0.95	5.65	12.53	6.88 ± 12.53
	Difetihalone	11	3.79	3.79	9.73	1.45	2.23	4.23	2.25	3.79 ± 2.25
	Flocoumafen	13	0.95	0.75	2.18	0.36	0.51	0.91	0.65	0.95 ± 0.65
	Total n=90	78.8% (n=71)	56.80	38.66	327.60	1.73	17.47	74.11	59.19	
Tawny Owl *Strix aluco*	Brodifacoum	15	26.24	19.60	103.2	2.8	4.30	31.74	30.11	26.24 ± 30.11
	Bromadiolone	14	57.51	13.90	401.33	1.53	3.78	71.32	105.12	57.51 ± 105.12
	Coumatetralyl	1	5.46	5.46	5.46	5.46	5.46	5.46	-	5.46 ± nan
	Difenacoum	5	2.88	1.59	5.65	0.74	0.89	5.49	2.48	2.88 ± 2.48
	Difetihalone	3	4.19	4.87	5.3	2.39	3.63	5.09	1.57	4.19 ± 1.57
	Total n=22	86.36% (n=19)	65.65	33.60	422.06	3.09	9.83	94.43	95.47	
Little Owl *Athene Noctua*	Brodifacoum	9	25.55	1.76	189.32	1.12	1.57	13.60	61.65	25.55 ± 61.65
	Bromadiolone	5	7.29	2.18	25.98	0.7	1.21	6.35	10.69	7.29 ± 10.69
	Difenacoum	1	0.45	0.45	0.45	0.45	0.46	0.46		0.46 ± nan
	Difetihalone	2	19.26	19.26	30.62	7.88	13.57	24.94	16.08	19.26 ± 16.08
	Total n=19	68.4% (n=13)	24.4	5.9	220.9	1.51	2.67	14-82	59.49	
	Brodifacoum	2	2.11	2.11	2.79	1.41	1.76	2.45	0.98	2.11 ± 0.98

Long-eared Owl *Asio otus*	Bromadiolone	1	10.69	10.69	10.69	10.69	10.69	10.69	-	10.69 ± nan
	Difetihalone	1	17.54	17.54	17.53	17.53	17.54	17.54	-	17.54 ± nan
	Flocoumafen	1	0.91	0.91	0.91	0.91	0.91	0.91	-	0.91 ± nan
	Total n=11	36.36% (n=4)	9.49	3.28	29.37	2.03	2.42	10.35	13.28	
Barn Owl *Tyto alba*	Brodifacoum	1	11.61	11.61	11.61	11.61	11.61	11.61	-	11.61 ± nan
	Bromadiolone	1	15.35	15.35	15.35	15.35	15.35	15.35	-	15.35 ± nan
	Flocoumafen	2	1.73	1.73	3.12	0.33	1.03	2.43	1.97	1.73 ± 1.97
	Total n=3	66.6% (n=2)	16.22	16.22	30.94	1.51	8.87	23.58	20.8	
Eurasian Scops Owl *Otus scops*	Brodifacoum	7	4.10	1.28	11.37	0.45	0.88	6.93	4.95	1.28 ± 4.95
	Bromadiolone	7	30.04	10.07	80.02	0.611	4.27	55.51	34.69	10.07 ± 34.69
	Total n=19	47.3% (n=9)	27.57	13.02	80.54	1.63	2.27	38.99	31.51	